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ERRATA

THE EFFECT OF THE TIME FACTOR ON THE AMOUNT OF PRESSOR MATERIAL PRESENT IN KIDNEY AFTER UNILATERAL LIGATION OF RENAL PEDICLE AND AFTER UNILATERAL LIGATION OF URETER[1]

JULIAN R. BECKWITH[2]

From the Biochemical Laboratory, University of Virginia, University

Increased amounts of pressor substance have been demonstrated in the kidneys of animals after complete (1) and partial (2) occlusion of the renal artery and also after ligation of the ureter (3). However, the effect of the duration of such occlusion or ligation has not been studied. It is the purpose of the present investigation to determine the relationship of the duration of *a*, complete ligation of the structures of the renal hilus, and *b*, occlusion of the ureter to the amount of pressor material present in the respective kidneys.

METHODS. In one group (a) of white rats, aged 3 to 4 months, the pedicle of one kidney was ligated, and in another group (b) one ureter was occluded. The animals in each group were sacrificed at varying intervals after the respective procedures. Extracts of both kidneys of each animal were prepared immediately after death by grinding the kidneys with the aid of powdered glass and putting the macerated tissues in 2 cc. of 2 per cent NaCl solution per gram of tissue and placing in a refrigerator for about fourteen hours. Then the material was adjusted to a pH of 4.5 and centrifuged. The supernatant fluid was tested for its pressor content by injecting it into white rats (weight 200–300 grams), anesthetized by 0.05 gram pentobarbital sodium per kgm. B. W., intraperitoneally. Blood pressure of these test rats was taken from a cannula in the carotid artery attached to a mercury manometer via a three way stopcock. One milligram of heparin (1 mgm. $=$ 110 units) in 0.1 cc. saline was injected before the observations were made and blood pressure readings were taken at
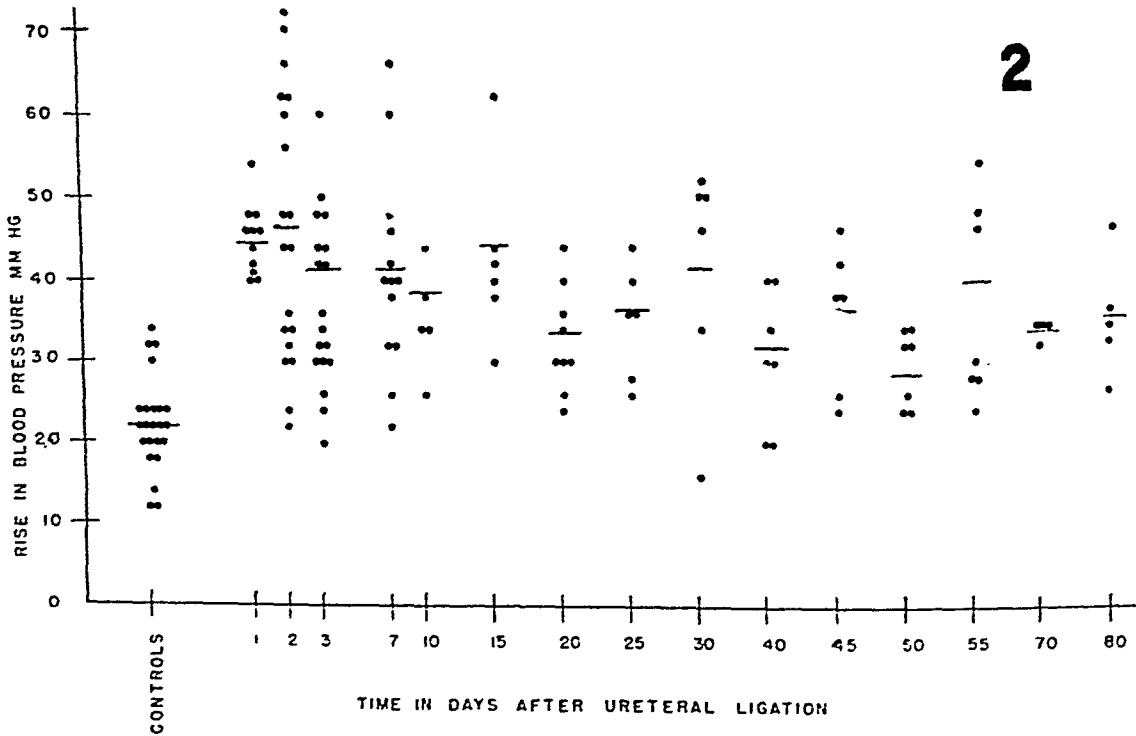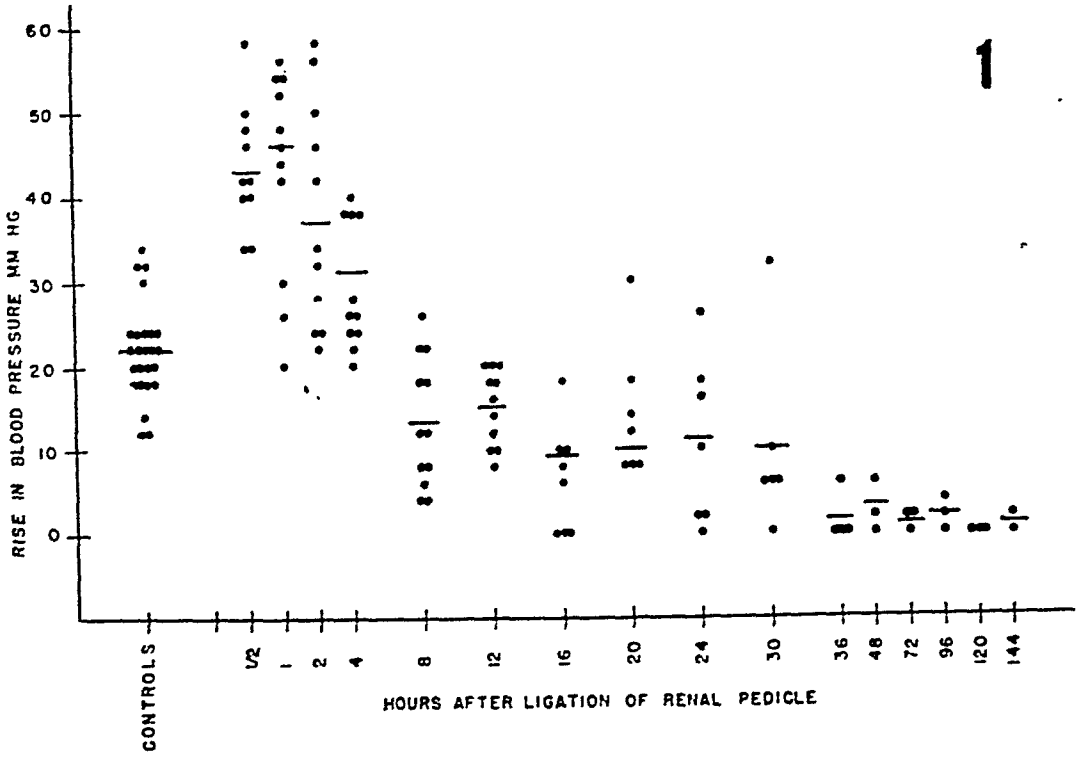
frequent intervals until a uniform pressure level was established. After stabilization of the blood pressure, 0.2 cc. of the extract to be tested was injected and blood pressure was taken every fifteen seconds for the first minute, every thirty seconds for the second minute and every sixty seconds for three additional minutes. The maximum increase in blood pressure above the basal level usually occurred in $1\frac{1}{2}$ to 2 minutes after injection and this figure was taken as the measure of pressor response.

RESULTS AND COMMENT.   a. *Unilateral ligation of renal pedicle* (fig. 1). The average rise in blood pressure produced by extracts of normal kidney was 22 mg. Hg. The average rise in blood pressure produced by extracts of kidneys removed $\frac{1}{2}$ and 1 hour after ligation of the renal pedicle produced an average blood pressure rise of 43 and 46 mm. Hg, respectively. This increase in pressor substance was presumably due to the fact that within the first hour, ligation of the renal pedicle caused ischemia of the kidney.

Extracts of kidneys removed more than 1 hour after ligation of the pedicle produced progressively less pressor response until extracts obtained more than 4 hours after ligation caused less average blood pressure elevation than did extracts of normal kidneys. A probable explanation of this observation would seem to be that enzymes after the first hour may inactivate the pressor substance.

b. *Occlusion of the ureter* (fig. 2). The normal controls used were the same as above. The greatest average blood pressure response in this group was 45 and 46 mg. Hg from extracts of kidneys removed 24 to 48 hours respectively after the ligation of the ureter. Ischemia of the kidney has been shown (4) to result shortly after ureteral ligation and presumptively this initial increase in pressor substance is due to ischemia of the kidney secondary to ureteral occlusion.

Extracts of kidneys with ureters ligated more than two days elicited progressively less blood pressure response. However, even 80 days after the ureter was tied, the kidneys contained amounts of pressor material greater than the average amount in normal kidneys. These kidneys were typically hydronephrotic and mere shells of tissue. Histologically, there was marked pressure atrophy and thinning of the cortex with round cell infiltration. The arteries appeared normal. Since there was increased pressor substance in the presence of atrophy of functional renal units, one might postulate that pressor substance was elaborated by some type of cell not involved in renal secretion. Such a postulation would be in accord with the work of Goormaghtigh and Grimson (5), who suggest possible endocrine cells in the kidney which secrete pressor material.

CONCLUSIONS

Kidneys removed within four hours after renal pedicle ligation contain greater than normal amounts of pressor substance. After this time the

amount of pressor substance decreases below that recorded for the control kidneys.

Amounts of pressor material in kidneys after ureteral ligation were greatest in the first two days after the procedure but remained in excess of normal in kidneys whose ureters were occluded for as long as eighty days.

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THE RÔLE OF THE HYPOTHALAMUS AND PREOPTIC REGION IN THE REGULATION OF HEART RATE[1]

S. C. WANG AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School

Since the discovery of the hypothalamus as a visceral center, numerous sympathetic functions have been ascribed to it (see review by Ranson and Magoun, 1939). But opinions differ as to whether cardiac acceleration also results from its stimulation. Jaegher and Bogaert reported in 1935 that the heart beats faster on stimulation of the hypothalamus; but no details were included. Kabat, Magoun and Ranson (1935) in their work on the vasomotor responses, could not obtain a uniform result on the heart, and they found only slight cardiac acceleration in 60 per cent of the cases in which stimulation brought about other sympathetic changes. But since the heart rate is definitely increased during emotional excitement and in the "sham rage" of decorticate animals (Cannon and Britton, 1925) and such spontaneous "rage" is known to originate from the hypothalamus (Bard, 1928), one would incline to believe that the hypothalamus takes an important part in cardiac regulation.

In our recent experiments with chloralose anesthesia, we have been confronted with numerous pressor reactions over 100 mm. Hg following the usual hypothalamic stimulation. Such high pressures were rarely obtained in our earlier experiments with sodium pentobarbital anesthesia. Since the previous work on cardiac rate was done on cats with the latter anesthesia it seemed worthwhile to repeat the experiments using chloralose as the anesthetic. The present report also includes a similar study on the preoptic region.

METHODS. Sixty milligrams of chloralose per kilo of body weight was administered intravenously into each of the 24 normal cats. The preoptic region and hypothalamus were stimulated in the usual manner (Kabat et al., 1935). A bipolar nichrome wire electrode, completely enameled except at the tips, was introduced into the desired region with the aid of the Horsley-Clarke stereotaxic instrument. The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 v) in the primary circuit with secondary coil at 9 to 9.5 cm.

The blood pressure was recorded from the right common carotid artery on a slowly moving kymograph. The speed of the drum was regulated so that the pulse could be easily recognized. The stimulus was given for a period of 30 seconds and the pulse was counted in intervals of 10 seconds, both before, during and after the stimulation. Various procedures, such as vagotomy, upper thoracic sympathetic ganglionectomy, adrenalectomy or tying of the abdominal vessels at the level of the diaphragm were carried out in some of the experiments and stimulation of the same region was then repeated.

The location of the reactive points was verified microscopically from a study of the serial sections stained by the Weil method.
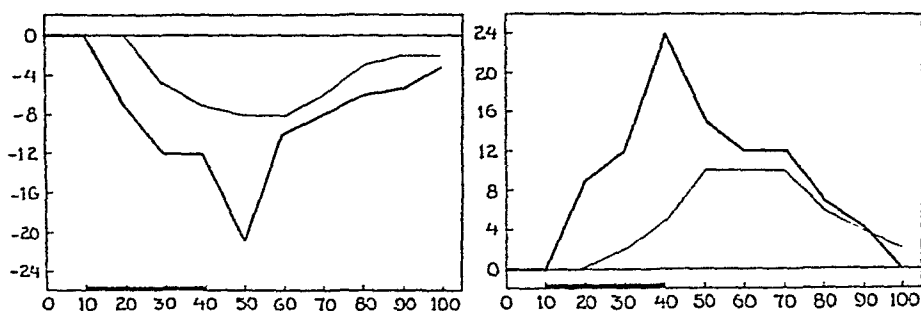
Fig. 1. Left. The effect of bilateral vagotomy on the cardiac slowing caused by stimulation of the preoptic region. Heavy lines, before vagotomy; light lines, after vagotomy. In this and other figures, ordinates denote the average per cent change of the heart rate per 10-second period; abscissae, time in seconds. The stimulus is marked at the bottom of these figures.

Fig. 2. Right. The effect of upper thoracic sympathetic ganglionectomy (from the stellate to T_5 ganglion) on the cardiac acceleration caused by hypothalamic stimulation. Vagi cut. Heavy lines, before ganglionectomy; light lines, after ganglionectomy.

RESULTS. Slowing of the heart with depressor reaction was observed in 6 cats when the preoptic region was stimulated. The lowering of the blood pressure during the 30 seconds of stimulation varied from 18 to 30 mm. Hg; the number of heart beats decreased 6 to 19 per cent. The maximal drop during the stimulus usually occurred in the third 10-second period (fig. 1). On withdrawal of the stimulus, the heart slowed down further with slight additional dip of the blood pressure level. In the vagotomized animals the depressor response was not appreciably altered but there was less slowing of the pulse. Nevertheless, the slowing was still considerable after vagotomy (fig. 1). The effect for a 30-second stimulus would last for 2 or more minutes.

Stimulation of the hypothalamus, on the other hand, uniformly yielded among other sympathetic discharges an average increase of heart rate of 5 to 25 per cent over the control. The maximal acceleration usually

appeared during the third 10-second period of the 30-second stimulus and in several instances reached as high as 35 per cent over the control. If the vagi were intact, there was frequently a sudden slowing of the heart rate below that of the control period on withdrawal of the stimulus. However, such findings were not universal. The acceleration would in general last for 2 minutes or more, particularly in vagotomized animals.

In 3 animals the hypothalamic stimulation was repeated after excision of the upper thoracic sympathetic chain from the stellate down to the fifth or sixth thoracic ganglion. There appeared no cardiac acceleration during the first 10-second stimulation and also very slight during the second 10-second period. The post-stimulus quickening of the heart was, however, almost as good as that before the extirpation (fig. 2). In 3 other cats both adrenal glands were tied so as to exclude them from the general
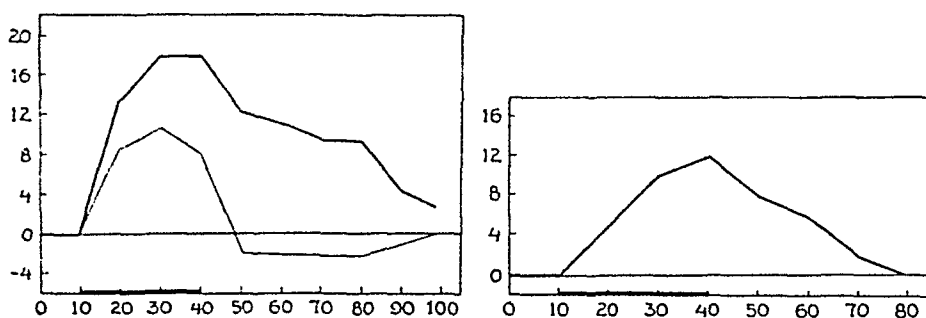
Fig. 3. Left. The effect of adrenalectomy on the cardiac acceleration caused by hypothalamic stimulation. Heavy lines, before adrenalectomy; light lines, after adrenalectomy.

Fig. 4. Right. Cardiac acceleration caused by hypothalamic stimulation after ligation of the abdominal vessels at the level of the diaphragm. No change of blood pressure was observed following the stimulation.

circulation. The cardiac acceleration lasted for a much shorter time after than before the adrenalectomy (fig. 3).

Since in all the experiments there was always a simultaneous occurrence of elevation of blood pressure and cardiac acceleration, a few experiments were performed on animals with the abdominal vessels, both the aorta and vena cava, tied at the level of the diaphragm (just above the right adrenal vein). In two of these animals, we were able to demonstrate cardiac acceleration on hypothalamic stimulation without accompanying arterial hypertension (fig. 4).

COMMENTS. The persistence of the depressor response to preoptic stimulation after vagotomy indicates that the major effect is not manifested through the vagi. Since the response in the form of bradycardia is less marked in vagotomized animals, it is fair to assume that the slowing of heart is partly vagal especially that part of the slowing that occurs after withdrawal of the stimulus. Our evidence suggests the possibility that

the preoptic region exerts a dual effect, stimulation resulting in excitation of the vagus and inhibition of the sympathetic.

Stimulation of the hypothalamus caused a sustained acceleration of the heart, both through the cardiac sympathetics and through the splanchnics and the adrenal glands. The former is largely responsible for the earlier effect, the latter for the late effect. No attempt has been made to correlate the degree of cardiac acceleration with different hypothalamic nuclei at various levels of the diencephalon. But, in general, there is an apparent correlation between the responses of the heart and that of the peripheral vascular system. Our evidence suggests that in normal animals maximal cardiac acceleration is only obtained in cases where there is a marked pressor reaction. On the other hand, we have shown that the former is not necessarily secondary to the increased arterial tension, as it can be demonstrated differentially in animals in which the rise in blood pressure has been prevented by ligation of the abdominal vessels.

In view of the low-frequency reversal of vasomotor, pupillary and respiratory responses reported by Hare and Geohegan (1939), it was of interest to see if a slowing of heart rate could be obtained on low-frequency stimulation of the hypothalamus. In 2 cats thus stimulated[2] (unpublished data), a marked depressor response of 40 mm. Hg was obtained at the frequency of 5 per second, where a pressor response was obtained previously and subsequently with a high-frequency stimulus. In each instance a slight increase of heart rate was obtained. Vagotomy did not influence the response.

SUMMARY

An average increase of heart rate of 5 to 25 per cent was demonstrated following a 30-second stimulation of the hypothalamus. This tachycardia is largely effected through the cardio-sympathetic nerves and adrenin, and can be obtained differentially without the accompanying hypertension in animals with the abdominal vessels tied at the level of the diaphragm.

Stimulation of the preoptic region resulted in an average slowing of the heart of 6 to 19 per cent. The effect is partly vagal.

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[2] Stimuli obtained from a thyratron regulated condenser-discharge.

THE EFFECT OF EPINEPHRINE ON THE POTASSIUM BALANCE OF THE HIND LIMBS OF THE FROG[1]

J. CLIFFORD STICKNEY[2]

From the Laboratory of Physiological Hygiene, University of Minnesota Medical School, Minneapolis

Since 1933 when Bachromejew discovered that the injection of epinephrine caused a rapid rise in the [K] of the blood of the cat, the researches of various workers have given us a well-defined picture of this phenomenal effect. D'Silva (7, 8) found in cats that the maximum [K] is attained in one minute after the injection, that this rapidly diminishes to the original level within about four minutes and may be followed by a considerable fall below the initial level within a further five minutes. A similar marked temporary rise in [K] followed by the fall to below the initial level was found in rabbits (18), in dogs (17) and in the human subject (1, 5, 6, 15). The exact time relations of the potassium response were found to vary with the species studied. We have concluded, on the basis of a series of experiments in which epinephrine was injected into sheep and goats, that these two species show the typical potassium response.

The increase in plasma potassium provoked by epinephrine is specific for that element since plasma sodium, calcium and ammonia remain essentially unchanged. The effect is seen with or without anesthesia, but in the latter case the effect is much greater.

Both D'Silva (8) on cats and Marenzi and Gerschman (17) on dogs gave convincing proof that the potassium in the response to epinephrine comes from the liver. In these studies the other organs of the body as well as the red blood cells were eliminated as producing the potassium.

The decrease in [K] following the rise is evidently due to the uptake of potassium by some organ or tissue. Marenzi and Gerschman (17) found that the muscles were active in this respect since the arteriovenous difference between the femoral artery and the femoral vein blood was found to be about +20 per cent one minute after the injection of epinephrine. A similar value (+20.67 per cent) was found by us in one out of two experi-

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[1] This report is from a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Minnesota.

[2] Present address: Department of Physiology, West Virginia University, Morgantown, W. Va.

ments in which the [K] of blood taken directly from the left ventricle of the unanesthetized dog by cardiac puncture was compared with that taken from the saphenous vein one minute after epinephrine. In a similar experiment on a dog under nembutal with the gastrocnemius muscle isolated the arteriovenous difference between left ventricle and femoral vein blood was +22.50 per cent at two minutes after epinephrine. In this latter case the possible uptake by bone or skin has been eliminated. In this connection, Houssay and Marenzi (13) found that the liver as well as the muscle gained potassium following the intravenous injection of KCl into dogs.

From the theoretical standpoint the most interesting phase of the potassium response to epinephrine is the after fall to below the initial concentrations. The initial rise seems to be the general result of a shocking agent, and our appreciation of that phase of potassium function is limited. But the after fall is paralleled by similar potassium changes occurring in connection with carbohydrate metabolism. Several investigators have reported the decrease in $[K]_s$ accompanying a similar decrease in $[P]_s$ during the assimilation of glucose (11). There is a like decrease in these elements following the administration of insulin (14, 16) during the time that the concentration of glucose in the plasma is being reduced. Furthermore it should be remembered that epinephrine itself normally plays a rôle in carbohydrate metabolism in mobilizing glucose from the liver during exercise, insulin hypoglycemia and emotional crises.

It seemed possible that the rapid disappearance and after fall of potassium might be due in part to the effect of epinephrine. Accordingly the following series of experiments in which this point might be tested were planned.

METHODS. 1. *Constant epinephrine in perfused hind limbs of frogs.* In order to maintain a blood supply having a constant [K] it was thought best to use the perfusion technique. Preparations of the isolated hind limbs of the double-pithed frog were used. The perfusion fluid was made up with the following composition: gum acacia 3.00 per cent; KCl, 0.650 per cent; $CaCl_2$, 0.050 per cent; glucose, 0.100 per cent; $NaHCO_3$, 0.068 per cent; Na_2HPO_4, 0.034 per cent; NaCl, 0.650 per cent. The pH was adjusted between 6.9 and 8.0. A perfusion pump (4) supplied pulsating pressure to the inflow cannula entering the terminal aorta. The outflow was collected from cannulae in the renal portal veins, all other egress being prevented by ligatures. [K] was determined (12) in arterial and venous samples collected at intervals during a period of $2\frac{1}{2}$ to $3\frac{1}{2}$ hours. Rates of flow were measured throughout. After a preliminary period of perfusion the fluid was changed to that containing 0.005 to 0.0025 mgm. epinephrine per cubic centimeter, and this was used throughout the remainder of the experiment (constant epinephrine). Two types of control

perfusion were done: one in which the perfusion was carried out as above except for the epinephrine (fast flow); the other in which the rate of flow was reduced to that found in the epinephrine perfusion, by reducing the perfusion pressure by half (slow flow).

The water lost by the perfusion fluid to the muscles was determined in a series of separate perfusions. The loss was determined on the bases of
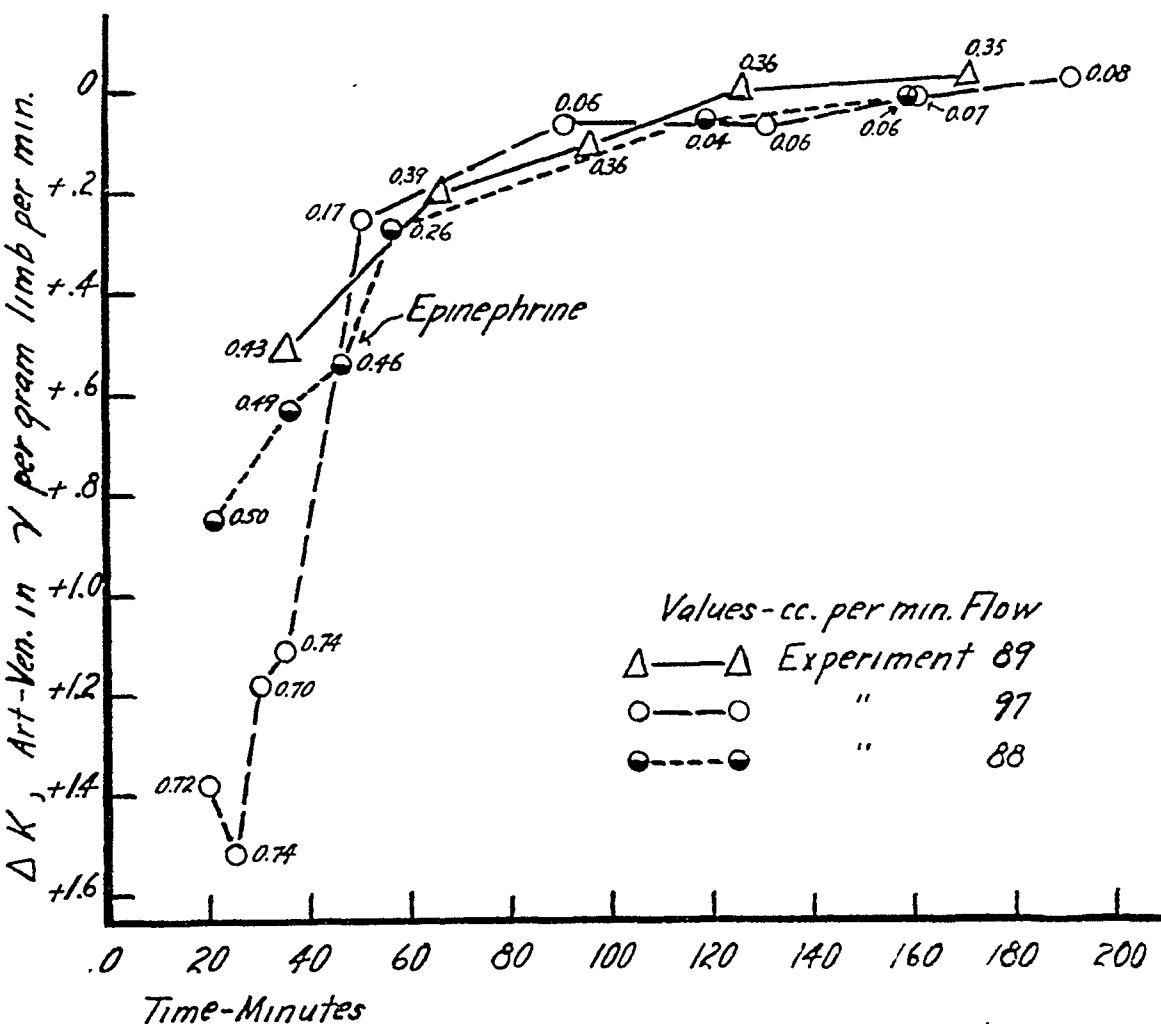
Fig. 1. Potassium exchanges in constant epinephrine (expt. 88), fast flow (expt. 89) and slow flow (expt. 97) perfusions of the hind limbs of the frog.

water contents of the fluid supplied to and the outflow from the hind limb preparations. In this series the water loss would not have caused an error of any significance.

2. *Potassium loss from stimulated frog muscles.* For these experiments the hind limbs of frogs were perfused with gum acacia-Ringer as described above. The muscles were stimulated indirectly by means of platinum electrodes in contact with the pelvic nerves in the abdomen. A Harvard

inductorium, the primary circuit of which was interrupted nine times a minute, was used.

In order to make certain that asphyxia or ischemia caused by low oxygen-content of the perfusion fluid were not factors two further experiments were carried out. In these the perfusion solution was approximately 4 per cent hemoglobin with the salt and pH content adjusted as before. The
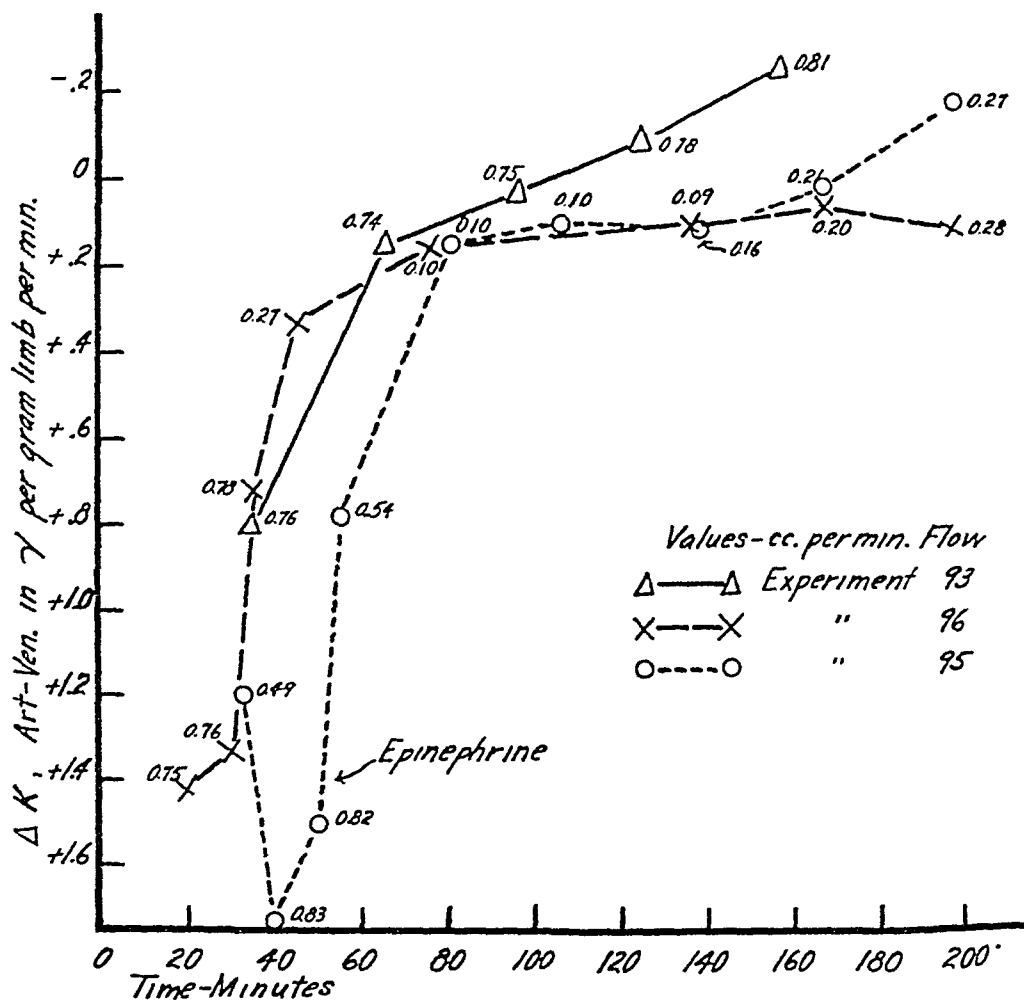
Fig. 2. Potassium exchanges in constant epinephrine (expt. 95), fast flow (expt. 93) and slow flow (expt. 96) perfusions of the hind limbs of the frog.

results were essentially the same as those obtained with the gum acacia-Ringer.

In two further stimulation experiments after a preliminary perfusion the perfusion fluid was changed to one containing 0.0025 mgm. epinephrine per cubic centimeter. The perfusion fluid was analyzed for potassium as before.

RESULTS. 1. *Constant epinephrine in perfused hind limbs of frogs.* The

results of five perfusions with constant epinephrine are shown in figures
1, 2 and 3. In the first two these are accompanied by the results of the
fast flow and slow flow perfusions without epinephrine. The K exchange
is shown graphically where the arteriovenous difference in gamma K per
gram of perfused hind limb per minute is plotted against the time from the
beginning of the perfusion. It will be noted that the [K] in the arterial in-
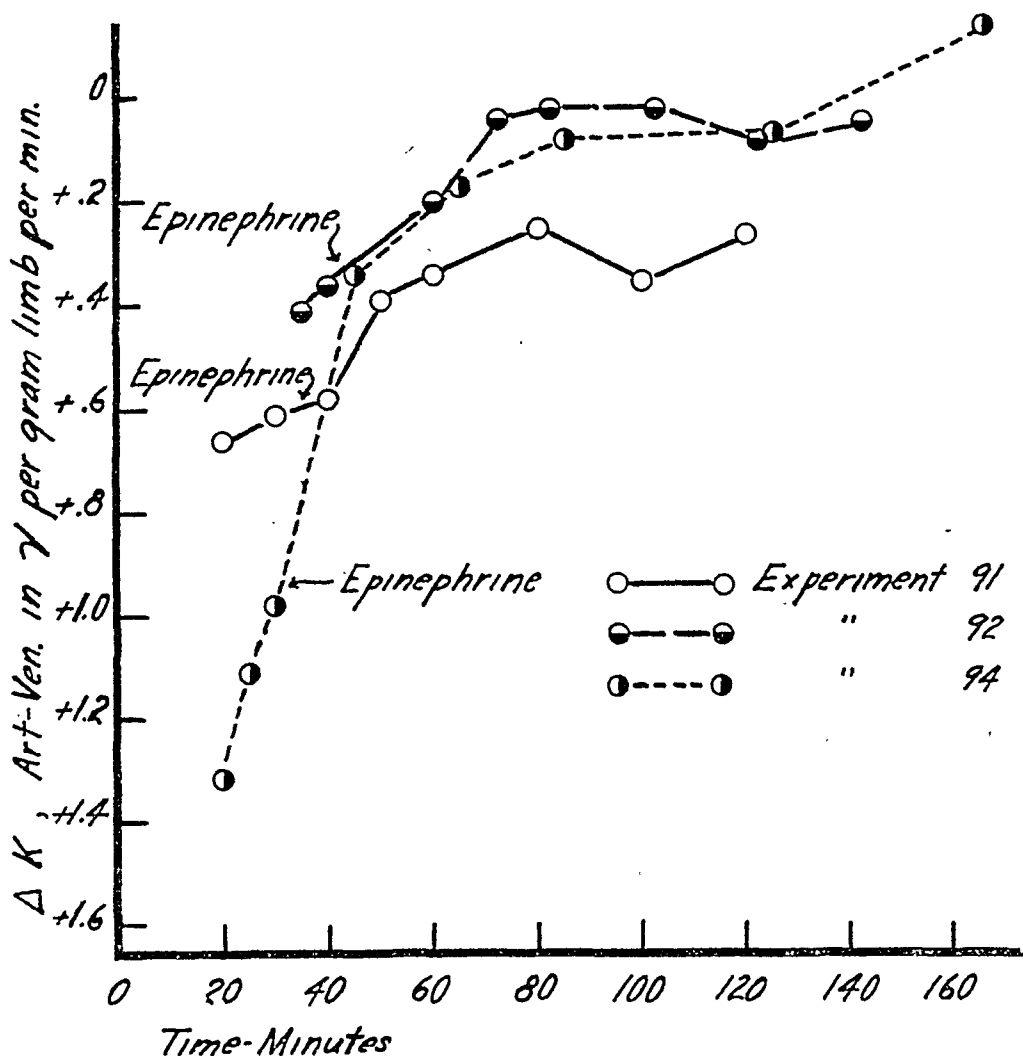
Fig. 3. Potassium exchanges in constant epinephrine perfusions of the hind
limbs of the frog.

flow was the most important factor in determining the direction and rate of
K exchange between the tissue and the perfusion fluid. The similarity
of the decline of the rate of exchange under the three different conditions
is very striking.

When the difference in [K] is plotted as in figure 4 it is evident that
epinephrine has a marked effect on the arteriovenous [K] difference.

(Fig. 4 is based on the same experiment as fig. 1.) This effect, however, does not appear to be specific for epinephrine for the slow flow perfusion shows it as well.

2. *Potassium loss from stimulated frog muscles.* In the experiments on the stimulated frog muscles the customary uptake of K was soon reversed and a loss of K by the muscle became evident. The results are plotted in
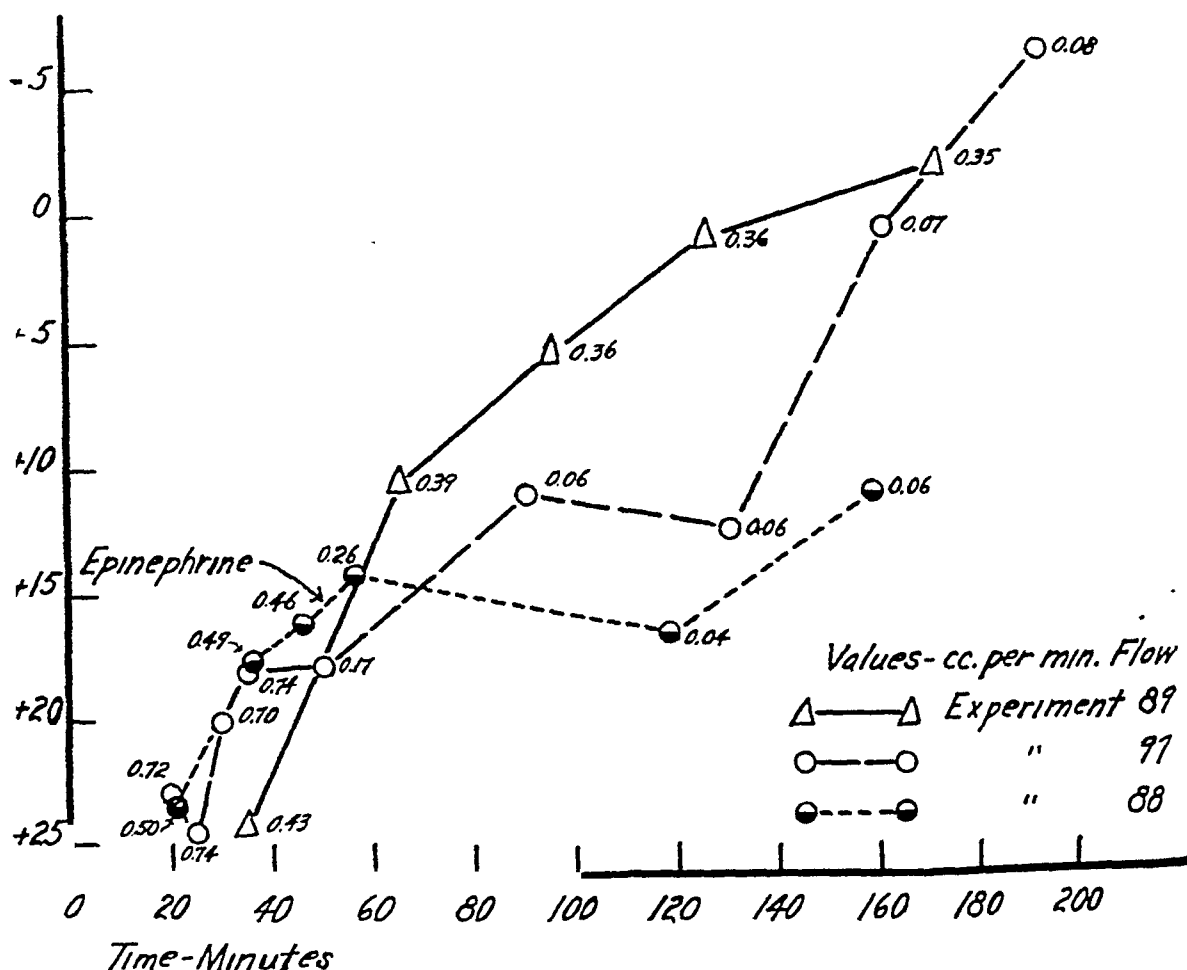
Fig. 4. Arteriovenous potassium differences in constant epinephrine (expt. 88), fast flow (expt. 89) and slow flow (expt. 97) perfusions of the hind limbs of the frog.

figure 5. The rate of K loss in both the fast flow and slow flow perfusions became quite constant at approximately 0.5 gamma K per gram of limb per minute. This rate of loss was maintained in each case until the experiments were terminated.

The effect of the constant perfusions with epinephrine in the two experiments in which it was used is also shown in figure 5. It will be noted that the K loss has been reduced in each case. The average magnitude of the reduction amounts to approximately 40 per cent.

DISCUSSION.   The effect of the reduction in the blood flow upon the $[K]_s$ has been studied in dogs by Baetjer (3) and by Fenn (10).   Their results, when recalculated to show actual exchanges, have been rather inconsistent, but have shown a reduction in the movement of potassium from tissue to
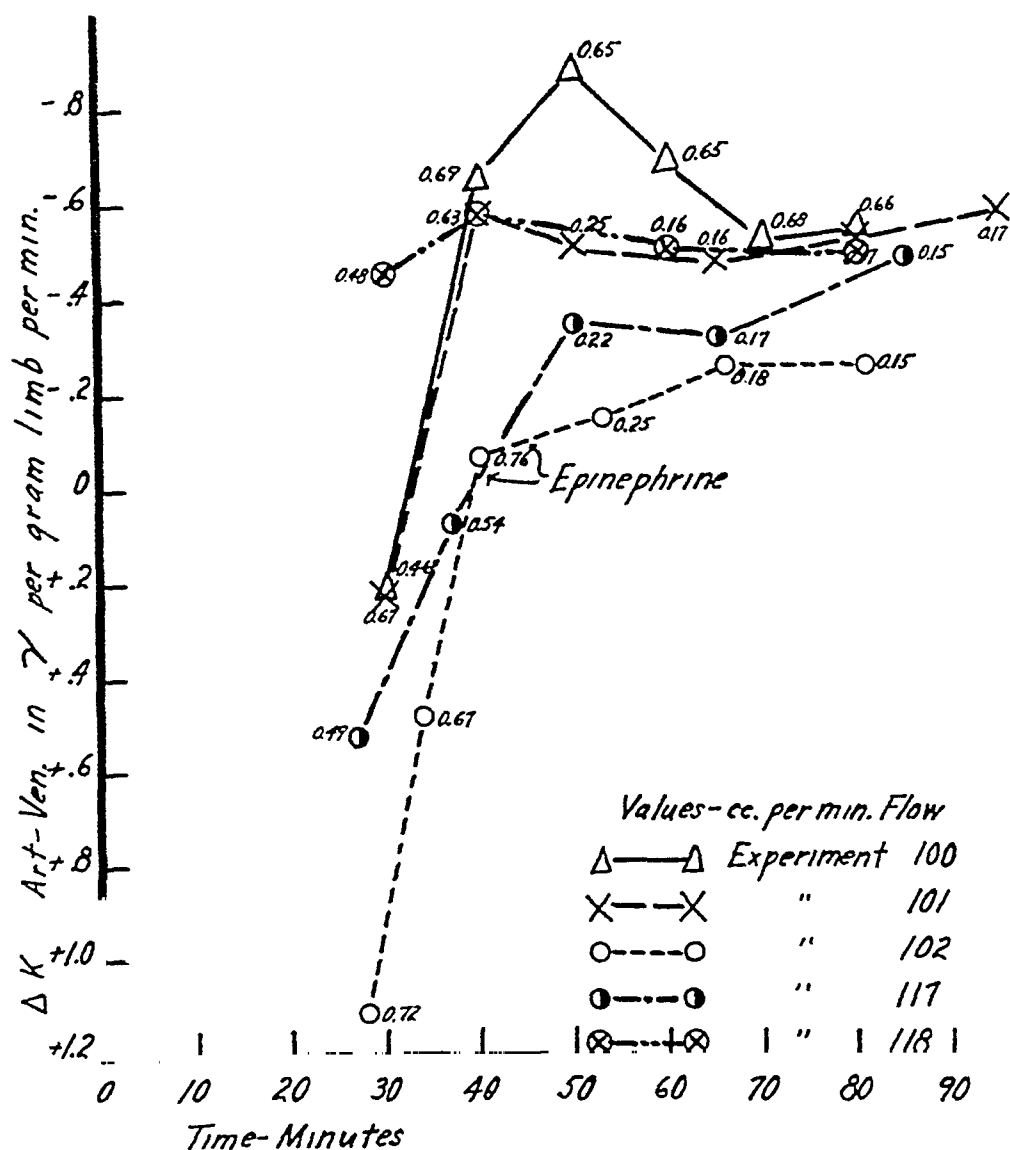
Fig. 5. Potassium exchanges in constant epinephrine (expts. 102 and 117), fast flow (expt. 100) and slow flow (expts. 101 and 118) perfusions of the hind limbs of the frog frequently stimulated.

vascular bed in most cases.   These experiments were done on warm blooded animals where asphyxia due to a reduced blood supply might complicate the results.

On the frog where the question of asphyxia has been ruled out, as has been described above, the results have been quite uniform throughout.

It has been demonstrated that the exchange of potassium is independent of the total rate of renewal of the blood phase. This was true whether the rate of flow was altered by epinephrine administration or by simple change in the perfusion pressure. Furthermore, a direct effect of epinephrine upon the potassium exchange or balance in resting muscle has not been demonstrated.

The results of the perfusions of the hind limbs frequently stimulated confirm the work of Fenn (9) on the frog. Fenn reported an average loss of 2.6 mM. potassium per 100 grams dry weight of muscle after 90 minutes' stimulation. If one assumes that the dry weight of the muscles of the leg are one-tenth the wet weight of the entire limb, which was measured in the present study, one arrives at a figure for potassium loss of 1.27 mM. per 100 grams dry weight of muscle per 90 minutes' stimulation. This is approximately one-half the loss reported by Fenn. The difference may be due to a number of things. In the present study the perfusion was not continued beyond one hour. Perhaps the most important difference was that in the experiments of Fenn intact frogs were used.

The potassium sparing action of epinephrine in active muscles presents a new problem. Before a very thorough understanding of the significance of this can be reached the mechanism of the usual potassium loss in muscular activity must be accounted for. A further clarification of the effect of epinephrine on the other phases of muscular activity would also help in interpreting these results.

SUMMARY

1. The typical rise and after fall in the plasma potassium concentration following epinephrine injection have been confirmed in the dog and have been found to be present in sheep and goats.

2. The active uptake of potassium by the muscles in this connection has been confirmed on dogs.

3. In the hind limbs of the frog perfused with gum acacia-Ringer the uptake of potassium from the perfusion fluid has been found to be independent of the rate of flow. Epinephrine has not been found to have a direct effect.

4. The hind limbs of the frog similarly perfused and indirectly stimulated 9 times per minute have been found to lose potassium at the rate of 0.5 to 0.6 gamma of potassium per gram of hind limb per minute.

5. If a constant perfusion of epinephrine is used with the stimulated muscles it has been found to reduce the rate of potassium loss by 40 per cent.

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THE ACTIVITY OF HEMOLYSINS IN VIVO

ERIC PONDER, CHESTER HYMAN AND LYMAN WHITE

From The Biological Laboratory, Cold Spring Harbor

There have appeared in the literature, from time to time, statements regarding the existence of hemolysins normally present in the blood stream, descriptions of their nature, and methods for their extraction. Thus Brinkman (1922) obtained from blood a lysin which he identified with linolenic acid, and Bergheim and Fahraeus (1936) extracted from horse serum a lytic substance similar to lysolecithin. Bogaert (1937) also obtained a lysin from serum (human), but could not determined its nature. Ponder (1921), Miller-Lamb (1927) and Abels (1934), have shown that there is a lysin present in normal urine, and presumably it is also present in normal blood. Recently Freeman and Johnson (1940) have demonstrated the presence of lysins (soaps) in chyle, and the work of Wigodsky (1940) has raised a question as to the effectiveness of indol *in vivo*. Ellis and his co-workers have obtained lysins from a number of tissues, and Griffin (unpublished work) has extracted a potent lysin from the pancreas.

In order to be able to form an idea as to whether such substances can act as intravascular lysins and so contribute to red cell breakdown in the animal, it is necessary to know something about the relation between the activity of a lysin *in vivo* and its activity as tested in the usual *in vitro* systems containing washed red cells in low volume concentration (1–5 per cent). In general, the activity of a lysin is less *in vivo* than *in vitro* for two reasons: 1. The volume concentration of the red cells upon which it has to act is greater (30–40 per cent), and there are powerful inhibitors present in the blood stream in the form of the serum proteins. 2. Lysins *in vitro* can react only with the red cells of the system, but lysins *in vivo* can react not only with red cells, but with the cells lining the blood-vessels, etc.; in this way the effectiveness of the lysins, so far as the red cells are concerned, is reduced. As opposed to these, there is the fact that a lysin operating *in vivo* may be continually produced and its concentration maintained, whereas in an *in vitro* system the concentration falls continuously to a theoretical zero. The estimation of the relative effect of these various factors is the subject of this paper.

1. *The effect of cell concentration and of inhibitors.* Taken separately, the effect of variations in the concentration of cell suspensions has been

studied by Ponder (1930), and the effect of the presence of varying amounts of serum by Ponder (1923, 1925) and by Ponder and Gordon (1934). By an extrapolation from their data, some idea of the combined effect can be arrived at, but a direct experimental determination is more reliable, and this involves plotting time-dilution curves for hemolysis in whole blood. This again involves being able to measure the end-point corresponding to some given degree of lysis, e.g., 100 per cent hemolysis, and it was not until we tried to do it that we realized how difficult it is. Ordinary opacimetric methods cannot be used because of the great optical density of whole blood, and conductivity methods, which we tried to use, give poor results because of the large stirring errors associated with any considerable degree of lysis. Finally we found that the usual technique (Ponder, 1934) can be used if a piece of thin-walled glass tubing, of 3 mm. internal diameter, is placed in each of the tubes containing the hemolytic

TABLE 1

SYSTEMS	ρ	x, γ/cc.
Saponin, washed cells...................................	0.006	15
Saponin, washed cells...................................	0.10	298
Saponin, washed cells...................................	0.35	1,060
Saponin, serum present..............................	0.35	3,800
Na taurocholate, washed cells........................	0.005	125
Na taurocholate, serum present.......................	0.36	30,400
Na oleate, washed cells...............................	0.006	46
Na oleate, serum present..............................	0.37	10,400

systems. By capillarity, the blood rises in the little tube, which is so narrow that the end-points corresponding to 100 per cent hemolysis can be easily determined. The little tubes can also be used to mix the systems.[1]

Proceeding in this way, we obtained results for the rabbit which are shown in table 1. This shows x, the quantity of lysin in γ/cc. required to produce complete lysis in 60 minutes in a number of typical systems. The volume concentration of the cells in each system is described by ρ.

The activity of a lysin such as saponin is only about $\frac{1}{250}$ as great in a system containing whole blood as in the usual test system containing washed cells in low concentration. For purposes of calculation one can say that the presence of serum and the high concentration of cells in whole blood reduces the activity of the lysin by a "factor of loss" of $\frac{15}{3800} = \frac{1}{250} = 4(10^{-3})$. For taurocholate the factor is $4.1(10^{-3})$, and for oleate it

[1] We have not been able to obtain satisfactory time-dilution curves with blood obtained from etherized animals. We kill the rabbit by a blow on the back of the neck, and then cut the carotids.

is 4.4(10^{-3}). For human blood the factors will have a similar value (Ponder, 1934). It is sufficient for our purpose if the order of magnitude of the factor is determined.

2. *Uptake of lysins by tissues.* The extent to which lysins are taken up by tissues can be found with sufficient accuracy by perfusing organs, such as rabbit kidney and heart, with varying concentrations of the lysins in Ringer's solution, and determining the concentration of lysin which appears from time to time in the perfusate by means of a hemolytic titration.[2] The lysin is prepared in varying concentrations (from 1 in 2,500 to 1 in 40,000 in the case of saponin) in Ringer's solution, and is contained in a bottle connected with a cannula inserted in the renal artery or in the aorta. Another cannula is placed in the renal vein or in the right side of the heart, and from this samples of the perfusate are taken from time to time. A second bottle, connected with the arterial cannula by a T-tube with stopcocks, contains Ringer's solution.

The organ is first perfused with Ringer's solution in order to remove red cells and plasma; this may take from 15 minutes to several hours. After this, a sample of the perfusate is taken, and the small amount of inhibitory substances present is determined by a hemolytic titration. The organ is then perfused with the solution of lysin for several hours, the perfusion rate being continuously observed and kept as constant as possible; from time to time, small samples of the perfusate are collected in vials, and the concentration of lysin in each is subsequently determined by hemolytic titration.

Irrespective of the organ perfused (we have used kidney, heart, and the entire animal, and have done 62 of these perfusion experiments in all), the results are substantially the same. If we plot y, the fraction of the initial lysin concentration removed by the organ, against v, the volume of fluid which has passed through the organ, we get a fairly good straight

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[2] A hemolytic titration is performed in the following way. A series of dilutions of the lysin, dissolved in Ringer's solution, are prepared, and systems of total volume 2 cc. are set up, containing 0.8 cc. of the various lysin dilutions, 0.8 cc. of Ringer's solution, and 0.4 cc. of a red cell suspension, prepared by suspending the thrice washed cells from 1 cc. of blood in 20 cc. of Ringer's solution. The mixture of lysin and Ringer's solution is brought to constant temperature in a water bath (described by Ponder, 1934), and the cells, also brought to constant temperature, are added. The time for complete hemolysis is measured, and if this is done for a number of dilutions of the lysin, a time-dilution curve results; this shows the relation between the dilution (or concentration in micrograms) of the lysin, and the time required for complete lysis. To find the lysin concentration in an unknown solution, such as a perfusate, one takes 0.8 cc. of the unknown, 0.8 cc. of Ringer's solution, and adds 0.4 cc. of the red cell suspension; the time for complete lysis is observed. Referring back to the time-dilution curve, this time corresponds to a time taken for complete lysis by a known concentration of the lysin; in this way the concentration of lysin in the unknown is immediately determined. The method is very precise, and can be modified in innumerable ways.

line in almost every case.   The equation of this line is $y = b - bv/a$, and the total amount of lysin which the organ can take up is, by an extrapolation which will at least yield a *minimum* value, $U = C_o \cdot ab/2$ mgm. when C_o, the initial concentration of lysin, is measured in milligrams per cubic centimeter and when v is measured in cubic centimeters.   The constant a is always a little less than unity, and b is the total volume in cubic centimeters which has passed through the organ when it has combined with so much lysin that it can combine with no more, i.e., when its lysin-binding powers are exhausted.   The graph of the results of an experiment on rabbit kidney perfused with 0.5 mgm./cc. saponin will make these relations clear (fig. 1).
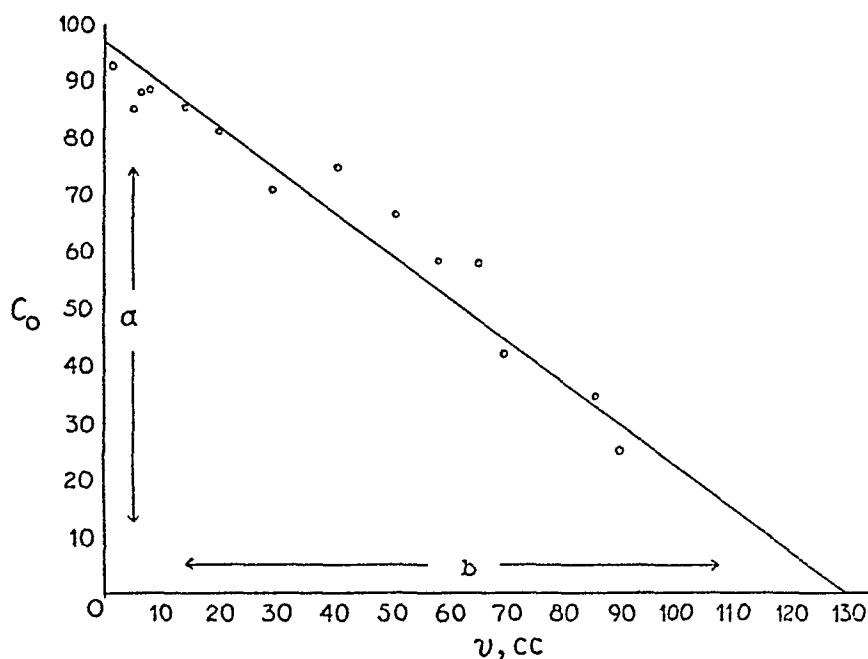
Fig. 1

The point of principal interest is the value of U, the total lysin-combining capacity of the organ, and table 2 shows some typical results in detail, together with the values of C_o, the initial concentration of lysin used in the perfusion fluid.[3]

In order to get an idea of the magnitude of the effects, take as typical the average of the values of U for the kidney perfused with saponin; this gives $U = 88$ mgm. per kidney or 13 mgm./gm. of kidney tissue. From table 1, the "uptake capacity," which must be of the same order as the amount of lysin required to produce complete hemolysis in an hour, is 3.8 mgm./cc. of blood, so, if we require to translate lytic activity in

[3] There may be a relation between C_o and U, but in our experiments it is obscured by variations in b, a constant which is affected by the perfusion rate.   This again is affected by the very variable degree of vaso-constriction, and tissue breakdown, which the lysins produce.

whole blood into lytic activity for whole blood passing through kidney tissue, we have to introduce another "factor of loss" of about 3.4 (10^{-1}), because the tissues through which the blood flows have such a great affinity for the lysin, and enter into competition for it.

3. *Constant production.* The equation which at least formally describes the action of lysins *in vitro*, where an initial concentration of lysin c is established, and is used up in reacting with the cells, is

$$dx/dt = k(c - x)^n, \dotfill (1)$$

whence, if $1/p = n$,

$$kt = \frac{p}{p - 1} \{c^{\frac{p-1}{p}} - (c - x)^{\frac{p-1}{p}}\} \dotfill (2)$$

and if $n = 1$, this becomes the well-known expression

$$kt = \log c/(c - x) \dotfill (3)$$

TABLE 2

ORGAN	LYSIN	C_o	a	b	U
Kidney................	Saponin	1.000	0.97	480	233.0
Kidney................	Saponin	0.500	0.92	180	41.4
Kidney................	Saponin	0.250	0.80	850	85.0
Kidney................	Saponin	0.125	0.85	270	14.3
Kidney................	Saponin	0.0625	0.75	2,840	66.6
Kidney................	Taurocholate	2.500	1.00	40	50.0
Kidney................	Glycocholate	2.500	1.00	105	131.0
Heart.................	Saponin	1.250	0.80	133	66.5
Entire rabbit..........	Saponin	0.250	0.95	6,640	789.0

If lysin is constantly supplied

$$dx/dt = kc \dotfill (4)$$

and

$$kt = x/c \dotfill (5)$$

To compare the effect of constantly supplying lysin as may occur *in vivo* (expressions 4, 5) with the effect of continually using up an initial quantity, as occurs *in vitro* (expressions 1, 2, 3), let us resort to a numerical example, using values for the constants which are generally found. Put $x = 20\gamma$ and $1/k = 200$ in (3); an initial concentration of 20.1γ will then bring about complete lysis in 1058 minutes. Put the same values in (5), and a constantly supplied quantity of $3.8\ \gamma$ will produce the same effect in the same time. The constant supply accordingly gives a "factor of gain" in activity of about 5.3. Since 1058 minutes amount to about a day, we can take the value of x which produces lysis in infinite time in the ordinary test system (the asymptote of the standard time dilution curve),

divide it by a "factor of gain" of about 5.0, and so obtain the amount of lysin which, if constantly supplied, would produce the same amount of lysis in a similar system per day.

4. *Conclusion.* In considering the relation of lytic activity *in vivo* to that in the usual test system we have these three factors: $4(10^{-3})$ as a result of the cell concentration and presence of serum inhibitors in the former system, $3(10^{-1})$ as a result of the completion of tissues for the lysin, and -5.3 as a result of a constant supply of lysins *in vivo*. The result is a "factor of loss" of $\frac{1}{170}$ or 0.006. If we observe the activity of a lysin *in vitro* by means of the usual test systems, we have to think of its *in vivo* activity, as producing comparable effects, as about $\frac{1}{200}$ of that observed. This means that any lysin hitherto extracted from blood, urine or tissue, and the activity of which has been determined *in vitro*, would, *in vivo*, be a very weak lysin indeed. Whether such a weak *in vivo* lysin, given sufficient time, would have an appreciable effect on the state of the red cells of the intact animal is a matter beyond the immediate scope of this paper.

SUMMARY

The lytic activity of a simple lysin *in vivo* compared to that in the usual *in vitro* test system involves three factors: the effect of the high concentration of cells and presence of serum inhibitors *in vivo*, the fact that lysins are taken up by the tissues *in vivo*, and the fact that lysins may be constantly supplied *in vivo*. Taking these factors together, the lytic activity of a lysin in the whole animal is probably only about $\frac{1}{200}$ that observed in the usual *in vitro* test systems.

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THE EFFECTS OF DENERVATION ON PHOSPHOLIPID ACTIVITY OF SKELETAL MUSCLE AS MEASURED WITH RADIOACTIVE PHOSPHORUS

H. D. FRIEDLANDER, I. PERLMAN and I. L. CHAIKOFF

From the Division of Physiology, University of California Medical School, Berkeley

As compared with tissues such as liver and small intestine, newly formed phospholipid makes its appearance very slowly in skeletal muscle. This has been shown with the aid of elaidic acid (1) and more recently with radioactive phosphorus (2, 3). In view of its bulk, however, muscle is an important depository of phospholipid. Thus it was found that about 36 per cent of the radioactive phosphorus incorporated into phospholipid by the whole bird appeared in relatively inactive tissues such as muscle, bone and blood within 12 hours after P^{32} injection (2).

It is shown in the present investigation that the normal rate of phospholipid activity of skeletal muscle depends upon its nerve supply. Denervation of skeletal muscle is followed by a profound increase in its content of labeled phospholipid synthesized in short intervals after the administration of radioactive phosphorus.

EXPERIMENTAL. The results recorded here were obtained from separate analyses of 144 rats, the data of part of which are shown in tables 1 to 5. Large adult rats, most of which weighed between 250 and 300 grams, were used. Denervation was performed by removing a small section of the sciatic nerve just below the trochanter while the rats were under light ether anesthesia. Radioactive phosphorus was injected subcutaneously as an isotonic solution of Na_2HPO_4; each rat received 1 cc. of this labeled phosphate solution. Muscles were removed at 2 intervals thereafter: 12 and 48 hours. Throughout this study analyses were made on a single group of muscles: the gastrocnemius and plantaris. These muscles can be rapidly removed together. Care was taken to ensure uniformity in sampling, the tendons and adhering tissues other than muscles being removed. The method by which the phospholipids were isolated and their radioactivity determined has been noted elsewhere (4). For the determination of total radioactive phosphorus, muscles were placed, with the usual precautions, in a muffle furnace maintained at 550° and the resulting ash transferred quantitatively by means of dilute HCl to blotting paper measuring 3 x 5 cm. The latter was dried over a hot plate,

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wrapped in thin cellophane and its radioactivity determined in the manner described for phospholipid (4).

RESULTS. *The content of labeled total phosphorus and of labeled phospholipid in corresponding normal muscles.* Before testing the effects of denervation, the uniformity of distribution of radioactive phosphorus and of radioactive phospholipid in similar muscles of the right and left legs of normal rats was determined. In table 1 the content of labeled phospholipid in the combined gastrocnemius and plantaris was compared in the right and left sides 48 hours after the subcutaneous injection of radioactive phosphorus. In 3 of the rats (168, 166 and 164) there was a close or fairly close agreement in the amount contained per gram of tissue in

TABLE 1

Phospholipid activity* of corresponding normal muscles 48 hours after P³² administration

RAT NO.	RIGHT LEG			LEFT LEG		
	Weight	Activity		Weight	Activity	
		Whole muscle $\times 10^2$	Per gram $\times 10^2$		Whole muscle $\times 10^2$	Per gram $\times 10^2$
	grams			*grams*		
160	0.95	0.68	0.71	0.91	0.87	0.96
161	1.13	0.65	0.58	1.11	0.73	0.66
162	1.35	0.87	0.64	1.36	1.07	0.79
163	1.54	0.74	0.42	1.44	0.97	0.60
164	1.27	0.49	0.39	1.26	0.48	0.38
165	1.43	0.96	0.67	1.31	0.99	0.76
166	1.23	0.92	0.74	1.28	0.97	0.70
167	1.55	0.99	0.64	1.53	0.82	0.54
168	1.36	0.90	0.66	1.38	1.02	0.73

* Measured as the per cent of the administered labeled phosphorus deposited as phospholipid.

both sides of the animal. Although differences were noted in the other animals, in no case did they exceed 42 per cent. The content of total labeled phosphorus in the combined gastrocnemius and plantaris is recorded in table 2. The values obtained per gram of tissue for right and left groups of muscles are in close agreement.

The effect of denervation on the content of labeled phospholipid of muscle. Radioactive phospholipid was measured in muscles at 2 intervals after injection of labeled phosphorus: 12 (table 3) and 48 hours (table 4). In the former, the muscles were examined at 12, 60, 108, 166, 204 and 300 hours after denervation. In muscles in which activity was determined 48 hours after P³² administration, denervation was performed 48, 120, 168, 216, 336 and 456 hours prior to their removal.

Twelve hours after denervation.   In this group of rats (table 3) the labeled phosphorus was injected immediately after denervation and right and left muscle groups examined 12 hours later.   In 4 of the 8 animals recorded, an increase of over 50 per cent in the labeled phospholipid content was observed in the denervated muscles as compared with those removed from the unoperated side.   At this short interval after denervation, however, a rise in the content of labeled phospholipid did not consistently appear, for in 3 of the animals an insignificant change was observed after section of the sciatic nerve.

Forty-eight hours after devervation.   Three rats received labeled phosphorus immediately after the sciatic nerve on one side had been sectioned and the phospholipid content of both gastrocnemii compared 48 hours later (table 4).   At this early interval after denervation, it was again

TABLE 2

*Total radioactive phosphorus uptake of corresponding normal muscles**

	RIGHT LEG			LEFT LEG		
RAT NO.		Activity			Activity	
	Weight	Whole muscle	Per gram	Weight	Whole muscle	Per gram
	grams			*grams*		
97	1.33	0.19	0.14	1.39	0.17	0.12
98	1.39	0.19	0.14	1.40	0.20	0.14
99	1.52	0.22	0.14	1.55	0.19	0.13
100	1.73	0.18	0.10	1.75	0.17	0.10

* Measured as the per cent of the administered radioactive phosphorus.   In this table the uptake of radioactive phosphorus 48 hours after its subcutaneous injection is recorded.

observed that although increases in labeled phospholipid were found in the 3 muscles whose sciatic nerves had been cut, the increase in only one of these could be regarded as significant.

Sixty hours after denervation.   A striking increase in the content of labeled phospholipid was observed in the denervated muscles of 11 of the 12 rats recorded in table 3.   Considerable variations were noted, but increases of over 100 per cent were obtained in 10 of the animals.   The increase of *over 500 per cent* in a single animal is indeed worthy of note.

One hundred eight to three hundred thirty-six hours after denervation.   In 35 animals the denervated muscles were compared with the intact muscles at 12 and 48 hours after the administration of P^{32}.   The results recorded in tables 3 and 4 leave no doubt that increases in the labeled phospholipid content occur in muscles between the intervals of 108 and 336 hours after denervation.   The increases for the *whole* muscle

TABLE 3

*Phospholipid activity of muscles measured 12 hours after P^{32} administration**

TIME AFTER DENER- VATION	RAT NO.	INTACT MUSCLE				DENERVATED MUSCLE				PER CENT CHANGE IN DENERVATED MUSCLE	
		Leg	Weight	Activity		Leg	Weight	Activity		Whole muscle	Per gram
				Whole muscle $\times 10^2$	Per gram $\times 10^2$			Whole muscle $\times 10^2$	Per gram $\times 10^2$		
hours			*grams*				*grams*				
12†	49	L	0.91	0.39	0.43	R	0.99	0.29	0.29	−26	−32
	50	R	1.11	0.26	0.23	L	1.17	0.26	0.22	0	0
	51	L	1.33	0.21	0.16	R	1.36	0.47	0.35	+120	+120
	52	R	0.75	0.17	0.23	L	0.72	0.32	0.44	+88	+91
	21	R	1.44	0.54	0.37	L	1.43	0.98	0.68	+81	+84
	22	R	1.77	0.38	0.21	L	1.78	0.81	0.45	+110	+110
	23	L	1.56	0.67	0.43	R	1.84	0.73	0.40	+9	+7
	24	L	1.57	0.40	0.26	R	1.51	0.57	0.38	+42	+46
60	25	L	1.98	0.20	0.10	R	1.85	1.22	0.66	+510	+560
	26	L	1.75	0.45	0.26	R	1.70	0.52	0.31	+16	+19
	27	R	1.57	0.30	0.10	L	1.53	0.85	0.56	+180	+460
	28	R	1.67	0.30	0.18	L	1.64	0.67	0.41	+120	+130
	9	R	1.02	0.36	0.35	L	0.97	1.32	1.36	+270	+290
	10	L	1.41	0.27	0.19	R	1.22	0.89	0.73	+230	+280
	11	R	1.31	0.43	0.33	L	1.27	0.65	0.51	+51	+55
	12	L	1.15	0.31	0.27	R	1.19	0.91	0.76	+190	+180
	5	R	1.81	0.47	0.26	L	1.73	1.14	0.66	+140	+150
	6	R	1.55	0.28	0.18	L	1.64	0.86	0.52	+210	+190
	7	L	1.70	0.27	0.16	R	1.57	0.78	0.50	+190	+210
	8	R	1.34	0.16	0.12	L	1.33	0.39	0.29	+140	+140
108	57	L	1.22	0.26	0.21	R	1.17	0.64	0.60	+150	+190
	58	R	1.07	0.52	0.49	L	0.93	0.82	0.88	+58	+80
	59	L	1.01	0.51	0.50	R	0.92	0.63	0.69	+24	+38
	60	R	1.06	0.37	0.35	L	0.89	1.06	1.30	+190	+270
166	37	R	1.60	0.27	0.17	L	1.56	0.45	0.29	+67	+71
	38	L	2.16	0.23	0.11	R	1.81	1.69	0.93	+630	+750
	39	L	1.45	0.34	0.23	R	1.19	1.51	1.27	+340	+450
	40	R	1.36	0.37	0.27	L	1.24	0.96	0.77	+160	+190
204	41	L	0.77	0.27	0.35	R	0.43	0.76	1.80	+180	+410
	42	R	1.10	0.24	0.22	L	0.89	0.71	0.80	+200	+260
	43	L	1.09	0.13	0.12	R	0.94	0.78	0.83	+500	+590
	44	R	1.09	0.11	0.10	L	0.91	0.36	0.40	+230	+300
300	45	R	1.20	0.17	0.14	L	0.91	0.43	0.47	+150	+240
	46	L	1.75	0.18	0.10	R	1.00	0.71	0.71	+290	+610
	47	L	1.17	0.38	0.32	R	0.85	0.92	1.10	+140	+250
	48	R	1.67	0.34	0.20	L	0.92	0.54	0.59	+59	+200

* Activity refers to the per cent of administered labeled phosphorus found as phospholipid.

† Phosphorus administered immediately after the nerves were cut.

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TABLE 4

Phospholipid activity of muscles measured 48 hours after P^{32} administration*

TIME AFTER DENER- VATION	RAT NO.	INTACT MUSCLE				DENERVATED MUSCLE				PER CENT CHANGE IN DENERVATED MUSCLE	
		Leg	Weight	Activity		Leg	Weight	Activity		Whole muscle	Per gram
				Whole muscle $\times 10^2$	Per gram $\times 10^2$			Whole muscle $\times 10^2$	Per gram $\times 10^2$		
hours			*grams*				*grams*				
48	54	R	1.12	0.46	0.41	L	1.09	1.43	1.31	+210	+220
	55	L	1.09	1.45	1.33	R	1.08	1.61	1.49	+11	+12
	56	R	1.32	1.46	1.03	L	1.29	1.63	1.26	+12	+22
120	29	L	1.61	0.91	0.57	R	1.45	2.21	1.52	+140	+170
	30	R	1.67	0.22	0.73	L	1.46	2.25	1.54	+84	+110
	32	L	1.62	0.96	0.59	R	1.39	2.10	1.51	+120	+160
	65	L	1.32	0.99	0.75	R	1.05	1.91	1.82	+93	+140
	66	R	1.14	0.60	0.53	L	1.01	2.36	2.34	+290	+340
	67	R	1.03	0.66	0.64	L	0.84	1.21	1.44	+83	+130
	68	L	1.08	0.52	0.48	R	0.91	2.10	2.30	+300	+380
168	184	L	0.72	0.84	1.12	R	0.78	1.96	2.60	+130	+130
	185	R	1.20	1.48	1.23	L	0.81	2.31	2.85	+56	+130
	186	R	1.05	1.24	1.18	L	0.78	2.26	2.90	+82	+150
	187	L	1.27	1.46	1.15	R	0.92	1.80	1.96	+23	+70
	188	R	1.15	1.23	1.07	L	0.83	3.00	3.62	+140	+240
216	69	R	0.89	1.22	1.37	L	0.60	2.88	4.80	+140	+250
	70	L	1.10	1.03	0.94	R	0.81	2.69	3.32	+160	+250
	71	R	1.07	1.67	1.56	L	0.77	3.00	3.89	+80	+150
336	61	R	1.52	0.41	0.27	L	0.94	1.21	1.29	+200	+380
	62	L	1.57	0.56	0.36	R	0.88	1.25	1.42	+120	+290
	63	R	1.16	0.56	0.48	L	0.59	1.21	2.05	+120	+330
	64	L	1.40	0.53	0.38	R	0.74	1.27	1.72	+140	+350
456	172	L	1.38	0.75	0.54	R	0.52	1.36	2.62	+81	+380
	173	R	1.40	1.01	0.72	L	0.50	1.35	2.75	+34	+280
	174	R	1.41	0.72	0.51	L	0.37	0.84	2.27	+17	+340
	175	L	1.30	0.98	0.75	R	0.50	2.38	4.76	+140	+530
	176	R	1.42	0.73	0.51	L	0.52	2.10	4.04	+190	+690
	177	R	1.44	1.41	0.98	L	0.99	1.78	1.80	+26	+84
	178	L	1.06	0.76	0.72	R	0.42	1.76	4.19	+130	+480
	179	R	1.14	0.95	0.83	L	0.47	2.30	5.05	+140	+510
	180	R	1.27	0.86	0.67	L	0.53	1.44	2.71	+67	+300

* Activity refers to the per cent of administered labeled phosphorous found as phospholipid.

varied from 24 to 630 per cent in table 3 and from 23 to 300 per cent in table 4. Since at these intervals after denervation weight loss had already

occurred, the increases measured on the basis of per gram of tissue are even greater. Forty-eight hours after P^{32} injection (table 4) these increases were 70 to 380 per cent, whereas 12 hours after P^{32} administration (table 3) 38 to 750 per cent increases were observed per gram of muscle.

Four hundred fifty-six hours after denervation. The longest interval after section of the nerve in which comparisons of the phospholipid content of muscle were made was 456 hours (table 4). At this interval, the atrophy of the denervated muscle was quite marked. A consistent increase in the content of labeled phospholipid was still found in the denervated muscle, a difference that amounted to as much as 690 per cent when comparisons were made per gram of tissue.

TABLE 5

Total radioactive phosphorus uptake of corresponding denervated and intact muscles

TIME OF MUSCLE EXCISION		RAT NO.	INTACT MUSCLE				DENERVATED MUSCLE				PER CENT CHANGE IN DENERVATED MUSCLE	
After denervation	After P administration		Leg	Weight	Activity		Leg	Weight	Activity		Whole muscle	Per gram
					Whole muscle	Per gram			Whole muscle	Per gram		
				grams				*grams*				
60	12	89	R	1.36	0.18	0.13	L	1.31	0.23	0.18	+28	+39
		90	R	1.00	0.20	0.20	L	0.91	0.26	0.29	+30	+45
		91	R	0.99	0.18	0.19	L	0.94	0.22	0.23	+22	+21
		92	R	1.34	0.25	0.18	L	1.24	0.31	0.25	+24	+39
96	48	93	L	1.60	0.23	0.15	R		0.22		0	
		94	L	1.00	0.19	0.19	R	0.83	0.19	0.23	0	+21
		95	L	1.00	0.21	0.21	R	0.80	0.21	0.27	0	+29
144	48	101	L	1.30	0.21	0.16	R	1.03	0.27	0.26	+29	+63
		102	R	1.45	0.25	0.17	L	1.12	0.32	0.29	+28	+71
		103	R	1.24	0.28	0.23	L	0.94	0.27	0.29	+4	+26
		104	L	1.12	0.23	0.21	R	0.85	0.27	0.32	+17	+53

The effect of denervation on the content of total labeled phosphorus of muscle. In three groups of rats (table 5) the denervated and intact muscles were ashed and their total labeled phosphorus content determined. The time intervals following denervation at which muscles were excised were 60, 96 and 144 hours. The first group was injected with labeled Na_2HPO_4 12 hours before sampling, while in the latter two groups this time interval was 48 hours. As shown in table 5, a small but consistent increase in total labeled phosphorus appeared as a result of denervation when the muscles are compared per gram of tissue. In no case did this increase exceed 71 per cent. When whole muscles are compared with each other, the differences between denervated and intact muscles often become

negligible. The changes in total labeled phosphorus resulting from denervation in general are small compared to those obtained for labeled phospholipid in which increases of over 200 per cent often occurred.

DISCUSSION. Denervation markedly altered the rate at which skeletal muscle deposited newly formed phospholipid. When compared with the corresponding muscle of the intact side, the denervated muscle frequently showed *increases of over 200 per cent* in its content of radioactive phospholipid. This effect was not uniform at early intervals, but by the time 60 hours had elapsed after section of one of the sciatic nerves, a consistent difference in the radioactive phospholipid between the 2 muscles made its appearance. This change in the capacity of the denervated muscle to deposit labeled phospholipid appeared before appreciable atrophy set in and was present for as long as 19 days after section of the nerve. It should be noted here in passing that only small changes—an increase—in the water content of the muscle occur after denervation (5, 6).

The relation between total phospholipid content of a muscle and its *use* has been investigated by Bloor (7, 8, 9), who points out that the "more-used" muscles contain higher amounts of total phospholipid than "less-used" muscles. It would therefore appear at first sight that disuse of a muscle brought about by denervation would decrease its phospholipid content. Although this has been said to occur by Cahn (10), Grund (11) reports no change in the phospholipid content of a muscle as measured on the basis of the fat-free dry tissue. It should be stressed, however, that the loss of voluntary motor control is not the only effect of denervation (12); hence an alteration in total phospholipid content of a muscle (or for that matter in the rate at which radioactive phospholipid is deposited) need not necessarily reflect the effects of disuse.

SUMMARY

1. The phospholipid activities of normal and denervated muscles were compared, with radioactive phosphorus as indicator.

2. Denervation was followed by a pronounced increase in the capacity of the muscle to deposit labeled phospholipid.

3. This change made its appearance before appreciable atrophy of the muscle set in and was still observed 19 days after section of the nerve.

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THE EFFECT OF HYPERPYREXIA ON THE SECRETION AND FLOW OF BILE

S. L. OSBORNE, F. S. GRODINS, L. GOLDMAN AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

This investigation was undertaken to determine the effect of hyperpyrexia on the formation of bile. Many investigators (1, 2, 3, 4, 7) have used heat in various forms including localized diathermy over the region of the liver in the belief that heat so applied produces a choleresis. No conclusive evidence has been presented to support the thesis.

METHOD. Experiments have been performed on anesthetized dogs with a temporary fistula and on unanesthetized dogs with a permanent bile fistula.

Anesthetized animals. Thirty-nine healthy dogs weighing from thirty to forty-five pounds were used. The animals were placed on a standard diet and were not fed for 18 hours prior to the experiment. They were anesthetized with intravenous sodium pentobarbital using 30 mgm. per kilo of body weight. A tracheal cannula was inserted. The common bile duct was cannulated close to the duodenum, and a tube brought through the abdominal incision dropping the bile into a suitable vessel. The cystic duct was ligated and the edges of the abdominal incision were held in apposition. Bile flow was recorded electrically concurrently with carotid blood pressure. Once regularity of bile flow was established for a period not less than 30 minutes, a control flow was recorded. After a suitable control period of 20 or 30 minutes, heat was applied and maintained until the dog's rectal temperature rose to 42°C. The Inductotherm was used as a source of heat. The inductance cable or electrode fashioned in the form of an ellipse was placed on the animal board and covered with burlap material to form a suitable dielectric spacing between cable and dog. The dog was tied to the board in the usual supine position. The heating period usually lasted one hour after which the dog's temperature was permitted to return to normal. This usually required several hours even though an electric fan was used to assist in cooling the animal. The average number of drops of bile per minute covering a ten-minute interval during the control period was taken as the control rate. After the temperature of the animal had been elevated, the average number of drops

per minute during the 10-minute period showing the fastest flow was selected as the maximum rate of bile flow during pyrexia.   The difference between this maximum and the basal control rate determined the rate of increase or decrease.   The increase or decrease in the rate of bile flow, or minute volume output, was based on the number of cubic centimeters collected per minute.   In this series we encountered two cases of acholia in which the liver would not even respond to intravenous sodium dehydrocholate.

Quantitative determinations for bile salts, cholesterol and bile pigment were made to ascertain whether or not there was a change in the biliary constituents.   Biopsy of the liver was made before and after the application of heat in three animals.

Experiments on unanesthetized animals with a permanent bile fistula. Nine female dogs weighing between twenty and thirty pounds were used. Under ether anesthesia the cystic duct was cannulated, the gall bladder removed and the common bile duct ligated and cut.   The rubber tubing from the cannula was coiled in the abdomen, led to the outside through a stab wound and attached to a rubber collecting bag.   Following recovery from the operation, which usually required an average of ten days, the dogs were placed on a standard diet.   The bile was collected and measured every 24 hours.   When the quantity of bile was constant within ± 10 to 12 per cent, the animal was considered ready for treatment.   The day prior to treatment bile samples were collected and measured at 8 a.m., 1 p.m., 3 p.m. and finally at 6 p.m.   Collections were made and measured at similar time intervals during the experiment.   Quantitative chemical determinations were made on the collected samples for cholic acid, bile pigment and cholesterol.

The dogs were placed in a cabinet which was heated by means of electric light bulbs.   An electric fan kept the air in circulation.   Air from the outside was introduced into the hot chamber through a rubber tube.   The temperature of the box was kept at approximately 45°C. to 48°C. until the dog's rectal temperature reached 105° to 107°F., which was maintained for a period of one to two hours.   This method of heating was chosen because of the difficulties encountered in using the high frequency current in heating dogs.   During the experiment the dog could be observed through a glass window in the lid of the cabinet.   Rectal temperature was taken with a Brown resistance recording thermometer.

RESULTS. *Experiments on anesthetized dogs.* Of the thirty-nine dogs used, the flow of bile was recorded by a drop counter in ten, and by the drop counter and volumetric method in eleven; nine served as controls; in seven the skin was heated excessively; the remaining two were acholic and their livers were not even stimulated with bile salts given intravenously.

Table 1 gives the results of twenty-two experiments as recorded by the kymograph tracing. Subsequent to the heating period, the entire record showed an increase in bile flow. The maximum rate in most instances occurred just after the maximum temperature had been obtained (fig. 1). As a rule, the rate of bile flow did not show an increase until the rectal temperature reached 41.5°C. At this stage the dog usually began to pant. All of the twenty-one treated dogs showed an increased rate of flow over the basal rate. This increase ranged from 12 to 500 per cent.

TABLE 1

Showing the effect of artificial pyrexia on anesthetized dogs during the ten-minute period of maximum increase in bile flow

DOG NO.	RECTAL TEMP. °C.				AVERAGE BILE FLOW DROPS PER MINUTE			
	Initial	Max.	Rise	Final	Initial	Max.	Final	Increase per cent
1	37.7	42	4.3	39.3	2.2	3.3	1.7	50
3	38.2	42.5	4.3	38.0	1.4	2.2	1.0	51
4	39.9	42.5	2.6	39.8	1.4	2.1	1.5	50
5	37.8	42.0	4.2	37.4	3.5	5.1	3.0	45
9	38.6	42.0	3.4	39.0	1.1	1.6	0.59	50
10	37.8	42.3	4.5	38.8	3.2	3.6	2.1	12
12	39.0	42.0	3.0	39.5	1.9	2.8	1.3	47
14	38.5	42.0	3.5		2.5	3.6	1.7	44
15a	38.5	42.0	3.5	39.5	1.7	10.2	3.4	500
15b	39.5	42.0	2.5	41.0	3.4	5.0	3.2	300
16	38.5	42.0	3.5	39.0	2.4	6.3	2.5	162
19	38.0	42.0	4.0	39.5	5.0	8.2	4.2	64
20	38.0	42.0	4.0	39.2	3.7	4.8	4.8	30
21	37.2	42.0	4.8	39.0	1.7	5.0	2.0	194
22	37.5	42.0	4.5	38.2	1.4	7.3	6.1	421.3
24	37.8	41.5	3.7	38.3	1.6	2.9	2.2	81.2
25	39.1	42.1	3.0	40.7	1.7	3.6	1.7	111.6
26	40.0	42.6	2.6	39.5	2.2	2.7	1.1	22.7
27	37.8	42.0	4.2	38.5	2.3	2.9	1.7	26.9
29	38.1	41.1	3.0	39.4	1.5	2.9	1.8	93.3
31	36.6	41.5	4.9	37.9	2.7	4.0	2.2	48.0
33	38.1	41.5	3.4	39.8	1.7	4.1	2.0	141.2

Dog 15a (table 1) showed a maximum increase of 500 per cent with a temperature elevation of 3.5°C. After cooling to normal, the animal was reheated 2.5°C. and this time the bile flow increased 300 per cent above the original control rate. All the dogs in which the skin was heated excessively with one exception either showed a decrease or else no change from the basal rate of flow.

During the course of the experiment, 5 to 10 hours, the bile flow frequently progressively decreased. This was shown by the results of the

control experiments. However, in 43 per cent of the treated animals the increased flow persisted after the temperature returned to its control level. The data in table 2 show that the average minute volume output increased in nine experiments from 13 to 100 per cent, whereas in two experiments a decrease in volume output occurred. The results of nine control experiments are also recorded in table 2.

Qualitative and quantitative chemical analysis of the bile was made on eleven treated and nine control animals. The summary of these data is included in table 2. Table 3 gives a typical protocol of an individual experiment.
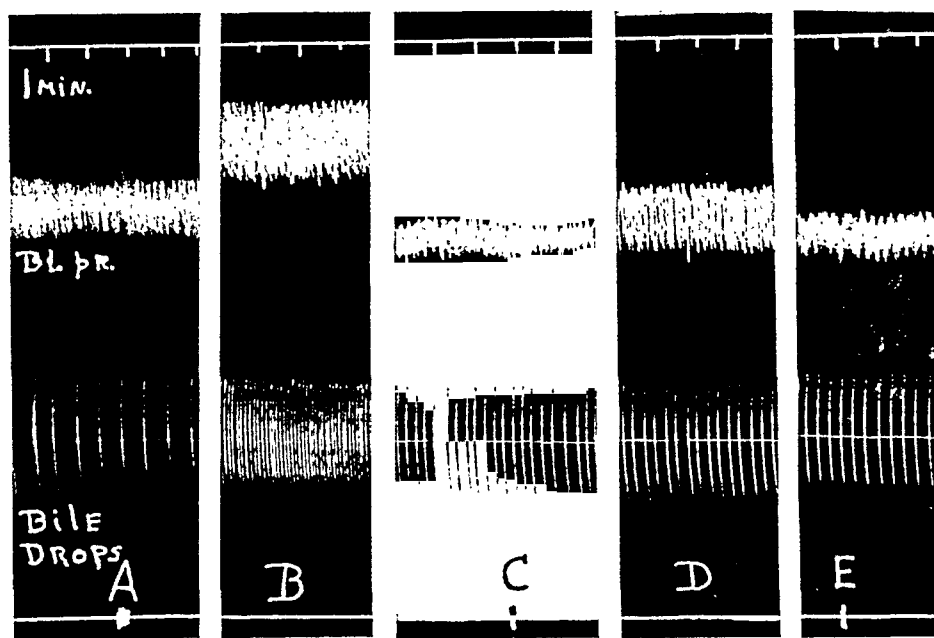
Fig. 1. Effect of hyperpyrexia on bile flow in a dog anesthetized with pentobarbital. *A*, control period; *B*, temperature 41.5°C.; *C*, temperature 39.0°C. three hours later than B; *D*, two hours after C; *E*, finish of experiment ten hours after control period.

We discovered that the rectal temperature of the control dogs did not remain constant under sodium pentobarbital anesthesia but continued to rise with time. This occurred even though an attempt was made to maintain basal temperature by the use of an electric fan. In seven of these dogs the temperature rose from 1° to 2.2°C. and was always maximal at the termination of the experiment.

The data on the chemical analysis of the bile for the treated animals show that a decreased minute output of cholic acid ranging from 13 to 70 per cent occurred in six animals while in three an increase occurred. Similarly, the cholic acid concentration was decreased in seven and increased in two. Five of the control dogs showed a decrease in the minute

output of cholic acid ranging from 10 to 64 per cent. The concentration of cholic acid was decreased in three, increased in one, and unchanged in one. In four of the control dogs we were unable to detect the presence of cholic acid in the last few specimens collected, although the analysis was repeated several times. Whether this was due to a cessation of the for-

TABLE 2

Quantitative and qualitative bile analysis

Eleven heated dogs, anesthetized

DOG NO.	TEMP. CHANGE	BILE VOL. (CC./MIN.)				CHOLATE OUTPUT (MGM. PER MIN.)			CHOLESTEROL OUTPUT (MGM. PER MIN.)			BILE PIGMENT OUTPUT (UNITS PER MIN.)		
		Total vol.	Control	During and after heat	Per cent change	Control	During and after heat	Per cent change	Control	During and after heat	Per cent change	Control	During and after heat	Per cent change
	°C.													
19	4.0	64.6	0.15	0.17	+13	3.31	2.34	−29.3						
20	4.0	40.9	0.14	0.12	−14.3	3.6	3.24	+10	0.918	0.025	+39			
21	4.8	32.3	0.07	0.10	+43	1.3	1.08	−16.9						
22	4.5	47.6	0.04	0.151	+77.5	1.23	2.49	+102.2				10.2	7.8	−23.6
24	3.7	23.8	0.055	0.088	+60	1.10	0.33	−70				1.3	1.1	−15.4
25	3.0	20.9	0.05	0.10	+100	1.44	1.60	+11.1	0.005	0.007	+40	6.78	9.0	+32.7
26	2.6	15.8	0.086	0.06	−30.3	0.87	0.43	−59.6	0.0017	0.0017	0	6.7	6.3	−6
27	4.2	26.4	0.058	0.08	+37.9	1.57	1.36	−13.4	0.0035	0.002	−70	5.7	5.6	−1.8
29	3.0	23.3	0.072	0.082	+13.7	2.6	1.14	−56.2	0.0099	0.0083	−8	1.68	5.2	+215
31	4.9	37.3	0.058	0.08	+37.9	Not secured			0.0079	0.0043	−45.5	5.7	4.7	−17.55
33	3.4	30.1	0.49	0.93	+89.7	Not secured						3.3	9.1	+175

Nine control experiments

DOG NO.	TEMP. CHANGE	TOTAL VOL.	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE
	°C.													
23	+2.2	35.2	0.044	0.087	+97.7	0.90	0.81	−10				5.4	7.3	+35
30	+1.4	15.6	0.050	0.040	−20.0	1.65	0.59	−64.3	0.0216	0.0067	−69	3.8	3.4	−10.6
32	+1.8	74.7	0.110	0.120	+9.1	Could not be read			0.0157	0.0068	−57	3.2	4.0	+25.0
34	+1.3	21.6	0.055	0.055	0	Could not be read			0.0035	0.0023	−34	6.2	8.8	+41.9
35	+1.7	5.6	0.023	0.018	−21.7	Could not be read						7.3	15.6	+113.0
36	+2.1	22.5	0.058	0.057	−1.7	Could not be read			0.0017	0.0015	−7	4.1	5.0	+22.0
37	+0.9	6.7	0.020	0.020	0	0.54	0.38	−29.6				11.8	16.1	+36.0
38	+0.6	6.9	0.025	0.017	−32.0	0.11	0.09	−18.0				1.5	3.8	+253.0
39	+0.2	48.5	0.130	0.110	−15.4	0.13	0.11	−15.3	0.0035	0.0023	−34	7.9	9.6	+21.0

mation of cholic acid by the liver or to a masking of the cholic acid test by large amounts of pigment could not be answered.

Four of the treated animals showed a decrease in the concentration of cholesterol while an increase occurred in two. The minute output of cholesterol was decreased in three, increased in two, and unchanged in

one. Five control dogs demonstrated diminished concentration and total cholesterol output.

Bile pigment determinations in the treated animals showed a decreased concentration and minute output in five and an increase in three. In eight of the controls, an increase in concentration and minute output of pigment occurred; a decrease occurred in one.

These results show that raising the body temperature of a dog anesthetized with pentobarbital from 3.0 to 4.9°C. increases the volume output of bile and when the results are compared with untreated controls usually decreases the output of bile pigment but has no significant effect on cholic acid and cholesterol output.

TABLE 3

Protocol of treated dog 22, anesthetized

Sample no.	BILE SAMPLE COLLECTION						BILE SALTS			CHOLESTEROL			BILE PIGMENTS	
	Sample		Collected		Total cc. collec.	No. of cc. per minute	Mgm./cc.	Total	Mgm./min.	Mgm./cc.	Mgm. total	Mgm./min.	Units per cc.	Units per minute
	Time	Temp.	Time	Temp.										
	min.	*°C.*	*min.*	*°C.*										
Control	0	37.3	75	38.5	3.0	0.040	30.9	92.7	1.23				768	10.2
2	75	38.5	55	41.9	3.3	0.062	22.7	7.49	1.36				640	11.6
3	130	41.9	60	40.8	3.7	0.060	23.4	86.6	1.44				685	11.4
4	190	40.8	30	40.0	5.0	0.166	22.1	110.5	3.68				310	10.3
5	220	40.0	30	40.0	4.5	0.150	21.4	96.3	3.21				250	8.3
6	250	40.0	28	39.2	6.7	0.239	17.1	114.6	4.09	0.085	0.5695	0.0203	146	5.2
7	278	39.2	42	38.9	9.4	0.224	10.8	101.5	2.41	0.072	0.6848	0.0163	142	3.4
8	320	38.9	50	38.2	12.0	0.240	12.6	101.2	3.02	0.078	0.9360	0.0187	131	2.6
Total 6 hours 10 minutes..........					47.6			828.3					3072	
Average during and after heat period.......					0.151				2.49					7.8

Experiments on unanesthetized "chronic" bile-fistula dogs. Twelve experiments were performed on nine dogs. Only those experimental data are shown in table 4 in which following the treatment the animal ate all of the diet.

In interpreting the data on volume output no change that is not greater than ± 10 per cent is significant because that is the daily experimental variation.

The 24 hour *volume output* was increased significantly in two of the twelve tests and only one of these may be considered physiologically significant (dog 1). In seven of the twelve no significant change occurred. A significant decrease in volume output occurred in three of the twelve tests. The greatest decrease, 47 per cent, occurred in dog 8 during the second

TABLE 4

A summary of the effects of artificial pyrexia on chronic unanesthetized bile fistula dogs

DOG	24 HOUR VOLUME OUTPUT (CC.)			24 HOUR CHOLIC ACID (GRAMS)			24 HOUR PIGMENT OUTPUT (MGM.)			24 HOUR CHOLESTEROL OUTPUT (MGM.)			REMARKS
	Control	Heat	Per cent change	Control	Heat	Per cent change	Control	Heat	Per cent change	Control	Heat	Per cent change	
1	122	167	+37	1.20	1.19	Insig.	85.4	54.3	−36	9.12	14.84	+62.5	Diathermy
2	221	255	+15	2.83	2.63	Insig.	77.3	88.2	Insig.	No data			Diathermy
3	131	122	Insig.	1.26	1.87	+48	228.3	545.2	+139	7.49	8.31	Insig.	Hot box
4	141	132	Insig.	2.14	2.35	Insig.	173.9	176.8	Insig.	14.90	15.68	Insig.	In excellent condition throughout. Hot box
5	290	232	−20	2.07	2.27	Insig.	103.0	105.2	Insig.	21.20	19.57	Insig.	In excellent condition throughout. Hot box
6	160	147	Insig.	1.84	2.75	+49	70.3	75.7	Insig.	7.2	7.8	Insig.	In excellent condition throughout. Hot box
7(1)	171	148	Insig.	2.96	3.12	Insig.	109	173	+58.6	No data			In excellent condition throughout. Hot box
7(2)	188	188	0	3.92	2.97	Insig.	138	124	Insig.	14.6	11.5	Insig.	Local diathermy over region of liver. In excellent condition
8(1)	212	204	Insig.	2.56	3.84	+50	77.8	86.3	Insig.	7.14	7.09	Insig.	In good condition throughout. Hot box
8(2)	192	101	−47	2.56	1.17	−54	75.5	56.5	−25	7.65	4.26	−44	Local diathermy over region of liver. Skin heated excessively
9(1)	111	85	−23	2.31	1.24	−46	41.3	41.9	Insig.	3.18	2.31	Insig.	In good condition throughout. Hot box
9(2)	130	126	Insig.	2.56	1.71	−20	53.4	53.5	Insig.	7.58	5.70	Insig.	In good condition throughout. Hot box

Insig. = Insignificant—i.e., within range of normal control variation.

test in which the skin was heated excessively. *In no experiment during the period of artificial pyrexia was the flow of bile increased, which is in contrast with the results on the anesthetized animals.*

Significant increases in *cholic acid* output occurred in three of the twelve tests. A significant change did not occur in six tests. A significant decrease occurred in three tests, two of which were associated with a decrease in volume output.

No significant changes occurred in *cholesterol* output except in the animal whose skin was overheated. In two tests the *pigment output* was significantly increased, in two it was decreased, and no significant change occurred in the remainder.

These results show that during the presence of artificially induced pyrexia in unanesthetized dogs a decrease in volume output of bile occurs, and that the effect of a 2- to 3-hour period of pyrexia has a variable but no appreciable physiologically beneficial effect on bile formation.

DISCUSSION. Methods available for the study of bile secretion in the experimental animal and in man are not entirely satisfactory. Acute biliary fistula experiments in dogs introduce the factor of anesthesia and are limited to observations over short periods of time. Much information has been obtained from studies on chronic biliary fistula animals but this method is difficult and requires accurate control of the spontaneous variations which occur and which unless properly controlled may be large. Much of the available data in the literature is of questionable significance because of the failure properly to consider spontaneous variations. In man, the methods ordinarily employed are only roughly quantitative and often do not distinguish between true hepatic secretory effects and motor effects, unless a fully recovered patient with a total bile fistula is used.

In man, Goldbruger (1) reported an increased flow of dark bile from a duodenal tube during and after the application of diathermy. The increase in flow may well have been due to motor effects, especially since Rafsky (2) found that local diathermy over the liver region often appeared to cause a contraction of the gall bladder. Frisch and Lasch (3) report four experiments on two patients using a duodenal tube. Although they found some increase in volume output as well as in the bilirubin and cholesterol concentration following diathermy in one patient, the data are inadequate to permit a definite conclusion.

Couperus and Moore (4) applied local diathermy to the liver of trained chronic biliary fistula dogs and reported an increase of 7 to 17 per cent in the twenty-four hour bile volume output. Long observation of biliary fistula animals in this laboratory (5, 6) has shown that these animals on a standard regimen may show as much as ± 20 per cent daily variation in volume output when the bag technique is employed and about ± 8 per cent with the suction method (5). It may be that the increased output

reported by these authors was within the limits of normal variation, particularly since only one twenty-four-hour control period was recorded. These workers drained the bile by means of a tube in the fundus of the gall bladder. Hence variations in the concentrating and motor activities of the organ must be considered.

Reinhold and Wilson (7) obtained a mild choleresis by the application of external heat (60-W. electric lamp) over the region of the liver. This was observed in animals under anesthesia.

In the present study we have used both acute and chronic bile fistula animals. In the acute (anesthetized) animals, there was a definite increase in bile volume output in the treated group, which is in agreement with the observations of Reinhold and Wilson. It has been shown (8) that heat applied to the liver area of anesthetized dogs increases hepatic blood flow, and Tanturi and Ivy (9) have reported that an increase in hepatic blood flow increases bile secretion in acute experiments on anesthetized dogs. It is most probable that the increase in bile volume output observed in our anesthetized animals was the result of an increase in blood flow to the liver. In this connection it should be pointed out that dogs under sodium pentobarbital anesthesia do not begin to pant until a temperature of 41.5°C. is reached. Gibson and Kopp (10) have shown that the marked decrease in blood volume which occurs with external heating devices is not observed when no sweating occurs. Hence there should have been no significant reduction in blood volume in these animals, a factor which if it occurred would tend to counteract the influence of heat in increasing the hepatic blood flow.

The results of the bile chemistry studies on these anesthetized animals were too variable to permit any definite conclusions of special significance to be drawn.

The unanesthetized animals with a permanent bile fistula showed no significant increase in bile volume output over the control level. Although apparently contradictory to the "acute" experiments, this result should not be unexpected. Hemingway (11) has shown and we have confirmed that unanesthetized dogs begin to pant when their temperature is elevated only slightly and one would, therefore, expect a marked decrease in blood volume to occur in these animals (Gibson and Kopp). The work of Reinhold and Wilson (7) indicates that hydremia favors choleresis. Conversely anhydremia may diminish bile volume output.

It has been shown (9) that stimulation of the hepatic sympathetic nerves causes a marked decrease in bile secretion, presumably by hepatic vasoconstriction with a reduction in liver blood flow. The excitement which occurs even in a well-trained dog when its rectal temperature is raised to 106–107°F. might easily produce sufficient sympathetic stimulation to inhibit bile secretion. Hence we have two factors operating in the

unanesthetized animals which were not present in the dogs under anesthesia; namely, oligemia and sympathetic stimulation. Both tend to inhibit the secretion of bile. This would account for the suppression of the secretion of bile during hyperpyrexia and the variable results for the 24-hour secretion.

As in the anesthetized animals, the results of the chemical studies in the unanesthetized animals are too variable to be of any physiological significance.

The question now arises, what can be expected to occur clinically in man. From what has been said above, it is apparent that there are several factors concerned and the results cannot be predicted with certainty. Thus there may be either an increase in bile secretion, a decrease, or no effect depending upon the relative importance of the controlling factors in any given case. For example, in a relatively phlegmatic individual where measures are taken to avoid excessive fluid loss one might reasonably expect an increase in bile secretion. On the other hand, in a highly nervous and excited patient, a decrease might be observed.

SUMMARY AND CONCLUSIONS

1. A choleretic effect was produced in anesthetized biliary fistula dogs as a result of hyperpyrexia.

2. In unanesthetized biliary fistula dogs, a significant choleresis was not demonstrated.

3. The probable cause of these diverse results is discussed.

4. No significant effect of hyperpyrexia on the output of the various constituents of bile was observed.

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A REËXAMINATION OF THE RÔLE OF THE STOMACH IN THE DIGESTION OF CARBOHYDRATE AND PROTEIN

J. M. BEAZELL

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

It is generally taught that the stomach is of little or no importance in starch digestion and, at least by implication, that it plays a relatively important rôle in the digestion of protein. There are a number of considerations which cast some doubt on the validity of this concept. The frequency of symptomless achlorhydria renders it apparent that the body can dispense with the proteolytic activity of the stomach without demonstrable ill effects. In four patients with achylia pancreatica Beazell, Schmidt and Ivy (1) found that failure of protein digestion closely paralleled that of fat, although all of the patients secreted free acid and were presumably digesting protein in the stomach at a "normal" rate. None of these patients, on the other hand, manifested any gross failure in starch digestion, which suggests that hydrolysis by ptyalin might be of greater importance than it is usually considered to be. Thus, in the human, it is observed that deficient pancreatic digestion but "normal" gastric digestion results in a marked impairment of protein digestion and absorption, while starch is relatively well tolerated. In view of these observations it appeared desirable to reëxamine the rôle of the stomach in the digestion of protein and starch.

Very little direct *in vivo* data on gastric digestion are available. Many authors have used the rate of evacuation of the stomach as an index of the rate of gastric digestion (2). However, although gastric digestion and gastric evacuation are related, they are not necessarily mutually dependent. Total gastric digestion in the normal human subject can not be determined. Such a determination necessitates a duodenostomy or duodenal fistula from which the gastric discharge can be collected as it is being evacuated through the pylorus. The only satisfactory alternative is to withdraw the gastric contents by means of a tube. This only yields information on the state of digestion at the time the samples were obtained, but what has gone before and what might be expected to follow can be reasonably surmised.

METHODS. In essence the experiment consisted of feeding normal

human subjects a meal of known nitrogen and carbohydrate content, withdrawing it from the stomach at the end of a specified period of time, and determining the ratio of "digested" to "undigested" nitrogen and carbohydrate in the recovered material. Since neither ptyalin nor pepsin characteristically reduce their respective substrates to absorbable products, the stage of degradation accepted as representing "digested" was chosen more or less arbitrarily. In the case of carbohydrate the production of reducing substances and in the case of protein the production of nitrogenous material soluble in 2.5 per cent tungstic acid were chosen as suitable end points of digestion. As used in this experiment, 2.5 per cent tungstic acid precipitates 47 per cent of the nitrogen in Bacto-pepton Difco (a commercial peptone preparation). Of the 53 per cent nitrogen remaining in solution only 22.8 per cent is in the form of ammonia and free amino acids. Thus, in addition to amino acids and ammonia, 2.5 per cent tungstic acid fails to precipitate many of the more complex products of protein digestion. Final proof that nitrogen soluble in 2.5 per cent tungstic acid constitutes a suitable end point for peptic digestion was obtained by an in vitro digestion experiment using U.S.P. scale pepsin. A 7.5 gram aliquot of the meal to be used was macerated, adjusted to pH 2.0, and digested with 100 mgm. of pepsin for 1 hour at 37°C. Under these conditions 37.3 per cent of the non-soluble nitrogen in the meal was digested to a form soluble in tungstic acid (preformed soluble nitrogen and that contributed by the pepsin being taken into consideration in the calculation). Throughout the following text nitrogenous material soluble in 2.5 per cent tungstic acid will be referred to as "soluble" nitrogen.

Eleven clinically normal young adult males were used as subjects for the experiment. In the initial phase of the study a test meal consisting of 150 grams of beef steak, 150 grams of potato, 50 grams of green peas, 10 grams of butter, and 200 cc. of water was employed. This meal was used in 2 experiments on each of two subjects who were capable of voluntarily regurgitating the gastric contents for analysis. The meal was masticated according to the normal habits of the subjects. Subsequently a finely divided homogeneous meal which could be completely evacuated from the stomach by means of an Ewald tube was employed. By resorting to the latter meal it was not only possible completely to evacuate the stomach (proven by x-ray control) but also to determine the dilution of the meal by gastric juice and saliva. As will be pointed out subsequently, this determination permitted interpretations which would not otherwise have been possible. The finely divided meal consisted of 100 grams of ground lean beef and 30 grams of potato starch boiled in sufficient water to yield a final volume of 400 cc. Twenty milligrams of phenolsulphonephthalein were dissolved in the water used in preparing the meal. The

dilution of the dye served to determine the dilution of the meal by the gastric juice and saliva.

The meal was ingested in the morning after a 14 hour fast.   Either 30 minutes, 1 hour, or 2 hours later the gastric contents were obtained either by regurgitation or by means of an Ewald tube.   The material recovered from the stomach was treated in the following manner.   One sample was set aside for the determination of free and total acid (using dimethyl-amino-azo-benzene and phenolphthalein respectively as indicators) and pH (glass electrode).   A second 30 cc. sample was added to a 5-minute old mixture of 15 cc. of 1.3 N sulphuric acid and 15 cc. of 10 per cent sodium tungstate.   After thorough shaking the mixture was centrifuged and the supernatant fluid was further purified by filtration.   This filtrate served for the determination of soluble nitrogen and reducing "sugar."   A third sample was analysed for total nitrogen and a fourth for total carbohydrate. In all experiments the composition of the meal as well as of the material recovered from the stomach was determined.   This was essential since the meal contained preformed reducing sugar and soluble nitrogen which had to be considered in determining the quantities of these substances produced in the stomach by digestion.

Nitrogen was determined by the macro Kjeldahl method and sugar by the hypo-iodite titration method (3).   Total carbohydrate was determined by the A.O.A.C. method (4).   Dilution was determined by the method of Wilhelmj et al. (5) as modified by Ivy, Schmidt and Beazell (6).   All analyses were performed in duplicate.

The reliability of the dye method for determining dilution in the presence of a mixed meal was tested in vitro.   It was found that a small, but measurable, quantity of the dye was adsorbed on the particulate material in the meal.   Since the ratio of particulate material to fluid would not be the same in the material recovered from the stomach as it was in the meal, it must be concluded that, as used in this experiment, the dye method yields dilution values somewhat smaller than they should be.   The error is not, however, of sufficient magnitude to confuse the interpretation in an experiment of this nature.   In view of the recent observations of Hollander et al. (7), it is worthy of note that we have found tungstic acid precipitation entirely satisfactory as a method for clearing the material recovered from the stomach of interfering substances prior to developing the color of the dye.

RESULTS.   The results obtained with the unground meal are summarized in table 1.   It should be emphasized that these results show only the state of digestion of the material remaining in the stomach.   That which had been evacuated into the intestine was perhaps less extensively hydrolyzed.   In this experiment it was not possible to calculate directly the quantity of digested material recovered from the stomach that repre-

sented preformed products of digestion in the meal. However, if it is assumed that the total carbohydrate and nitrogen left the stomach at the same rate as the "digested" carbohydrate and nitrogen, this factor can be taken into consideration in the calculation. The values in the table listed under the heading "per cent digested" were calculated on this basis. For example, if 10 per cent of the total nitrogen of the meal was in the form of soluble nitrogen, then it was assumed that 10 per cent of the total nitrogen recovered from the stomach was preformed soluble nitrogen, and in the calculation, this quantity was subtracted from the quantity of

TABLE 1

Gastric digestion of protein and carbohydrate normal meal

SUBJECT	MEAL						GASTRIC CONTENTS							
	Total nitrogen	Total soluble nitrogen*	Total carbohydrate	Total reducing sugar†	Per cent total nitrogen in form of soluble nitrogen	Per cent total carbohydrate in form of sugar	Volume recovered	pH	Total nitrogen	Total soluble nitrogen	Total carbohydrate	Total reducing sugar†	Per cent nitrogenous material digested in stomach‡	Per cent carbohydrate digested in stomach‡
1 hour period														
J. W.	5.6	0.84	42	7.56	15	18	175	4.5	1.73	0.175	6.3	4.13	0	47.7
H. A.	5.6	0.87	30.6	5.88	15.6	19.2	175	3.4	1.27	0.143	5.6	3.06	0	35.4
Ave.	5.6	0.86	36.3	6.72	15.3	18.6	175	4.0	1.50	0.159	5.9	3.59	0	41.5
0.5 hour period														
J. W.	6.75	0.90	34.3	4.85	13.3	14.2	215	5.1	2.12	0.200	8.6	4.98	0	43.8
H. A.	6.75	0.90	34.3	4.85	13.3	14.2	360	4.4	4.16	0.96	14.4	7.20	8.9	35.8
Ave.	6.75	0.90	34.3	4.85	13.3	14.2	287	4.7	3.14	0.58	11.5	6.09	4.5	39.8

* Soluble in 2.5 per cent tungstic acid.
† As maltose.
‡ Corrected for preformed digested material contributed by meal.

soluble nitrogen actually recovered from the stomach. The assumption that the "digested" and "undigested" fractions of the meal leave the stomach at the same rate is admittedly open to question. However, the error in interpretation would be greater if the factor of preformed "digested" material were neglected. Further, the digestion values compare favorably with those obtained in the subsequent experiments in which the preformed products of digestion in the material recovered from the stomach could be accurately determined (vide infra).

In only one of the 4 experiments of this series was the protein digested

to a measurable extent during the 30 or 60 minute period that it remained in the stomach. An important fraction of the carbohydrate, on the other hand, was hydrolyzed in every case. It is interesting to note that the digestion of the carbohydrate was not significantly further advanced at the end of 1 hour than it was at the end of 30 minutes. This might be anticipated since the activity of ptyalin diminishes rapidly as the pH falls.

The results obtained with the finely divided meal are summarized in table 2. Here again the values represent the state of digestion of the material remaining in the stomach at the end of 1 or 2 hours. Since in this experiment the stomach was completely evacuated and the dilution of the dye was determined, it was possible to calculate accurately the total quantity of the meal that had been evacuated into the intestine, and the fraction of the total digested material recovered from the stomach that represented predigested products in the meal. For example: if the meal contained 0.500 gram of soluble nitrogen and 50 per cent of the original meal was still present in the stomach at the end of the hour, then of the total quantity of soluble nitrogen recovered from the stomach, 0.250 gram would represent preformed soluble nitrogen in the meal. In a similar manner the theoretical starch content of the material recovered from the stomach was calculated.

Again in this experiment the quantity of nitrogenous material digested in 1 hour to products soluble in tungstic acid was insignificant (average 2.5 per cent) while an important fraction of the carbohydrate was digested to reducing sugar (19.6 per cent). When the period of time the meal was permitted to remain in the stomach was extended to 2 hours, the quantity of nitrogenous material "digested" was increased to an average of 9.5 per cent.

DISCUSSION. On first examination the interpretation of these results appears to be open to criticism in the three respects listed below. It is felt, however, that all three criticisms can be convincingly refuted.

I. Since the optimum pH for pepsin activity is in the neighborhood of 2.0, it might be argued that a 1 hour digestion period does not permit the development of sufficient gastric acidity to fully activate the pepsin. However, it will be noted in table 2 that by the end of 1 hour, in all cases except two (H. W. and J. V.), more than 50 per cent of the nitrogen (average 69.1 per cent) had been evacuated from the stomach into the intestine. In other words, a meal of this type is largely evacuated from the stomach before significant protein digestion can occur. That subsequent more complete hydrolysis of the nitrogenous material remaining in the stomach at the end of an hour does occur is shown by the fact that when the digestion period was extended to 2 hours an average of 9.5 per cent was reduced to a soluble form. This value, representing the digestion of less than 50 per cent of the protein of the meal, would not support the concept that

the stomach is an important site of protein digestion. In the case of carbohydrate, on the other hand, maximum digestion occurs early (table 1), probably preceding the period at which gastric evacuation has assumed significant proportions.

TABLE 2

Gastric digestion of protein and carbohydrate finely divided meal

SUBJECT	VOLUME RECOVERED FROM STOMACH	VOLUME RECOVERED CORRECTED FOR DILUTION	ACIDITY		pH	COMPOSITION OF MEAL		COMPOSITION OF MATERIAL RECOVERED FROM STOMACH					PER CENT STARCH DIGESTED TO SUGAR	PER CENT NITROGENOUS MATERIAL DIGESTED IN STOMACH	PER CENT INGESTED NITROGEN EVACUATED INTO INTESTINE
			Free	Total		Total nitrogen	Total soluble nitrogen	Total nitrogen	Total soluble nitrogen*	Total sugar*	Starch equivalent of recovered sugar	Calculated starch of recovered material			
1 hour period															
J. W.....	230	197	22	48	2.6	3.04	0.34	1.23	0.037	4.27	4.00	14.8	27	3.0	59.8
R. M.....	230	144	0	23	3.5	2.98	0.31	0.93	0		2.05	1.92	10.8	17.8	0	69.0
J. V......	350	292	11	52	2.8	2.45	0.34	1.79	0.070	4.16	3.80	21.9	17.6	3.9	27.0
G. L.....	270	211	33	100	1.4	2.63	0.30	0.70	0.013	2.99	2.80	15.8	17.8	1.9	73.5
V. S......	215	176	30	84	1.7	2.63	0.30	0.64	0.002	2.35	2.20	13.4	14.4	0.3	75.8
H. W.....	440	366	31	76	1.8	2.45	0.34	1.63	0.010	4.70	4.40	27.5	16.0	0.6	33.4
W. L.....	340	188	16	55	2.5	2.39	0.30	0.71	0.047	4.21	3.93	14.1	27.8	6.6	70.0
M. B.....	58	38	24	73	2.1	2.39	0.30	0.19	0.013	0.76	0.71	2.9	24.4	6.7	92.0
Ave....	267	202	21	64	2.3	2.62	0.32	0.98	0.024	3.19	2.98	15.2	19.6	2.45	62.5
2 hour period															
J. W.....	45	29.3	0	37	3.8	2.85	0.24	0.33	0.031	0.364	0.340	2.20	15.4	9.3	89
R. M.....	100	79.0	21	56	1.9	3.26	0.27	0.28	0.018	0.387	0.360	5.90	6.1	6.5	92
H. W.....	130	45.8	25	72	1.8	2.80	0.25	0.22	0.025	0.465	0.435	3.45	12.6	12.1	92
J. C......	140	140.0	35	108	2.0	3.32	0.28	0.83	0.086	1.960	1.830	10.5	17.5	10.3	75
T. K.....	100	72.8	30	92	1.7	3.32	0.28	0.28	0.024	0.940	0.880	5.47	16.1	8.8	92
Ave....	103	73.4	22.5	73.0	2.2	3.11	0.26	0.39	0.037	0.823	0.769	5.50	13.5	9.5	87.5

* Corrected for preformed digested material contributed by meal.

II. It might also be argued that the stomach would selectively evacuate the products of digestion leaving behind only the undigested residue. However, the soluble products of digestion would leave the stomach no more rapidly than the equally soluble dye and, in the second series of experiments, all interpretations are influenced by the quantity of dye remaining in the stomach at the end of the 1 hour period.

III. It is generally accepted (8) that protein hydrolysis by pepsin does

not proceed to a significant extent beyond the production of peptone. Therefore nitrogen soluble in tungstic acid (which precipitates approximately 50 per cent of the nitrogen in a relatively pure peptone preparation) might be considered too far advanced in the hydrolytic series to represent a suitable end point of pepsin hydrolysis. However, it was shown (vide supra) that in a period of 1 hour under optimal conditions, pepsin is capable of reducing 37 per cent of the nitrogen of the standard meal to a form that is soluble in tungstic acid. It might be considered, then, that although pepsin is potentially a potent proteolytic agent, conditions in the stomach are not such as to permit it to operate effectively. Further, so far as comparing the relative importance of protein and starch digestion is concerned, reducing sugars are further advanced in starch hydrolysis than tungstic acid soluble nitrogen is in protein hydrolysis.

Since, during the period the meal remained in the stomach before being withdrawn for analysis the extent of the digestion of that fraction which was evacuated into the intestine was undetermined, the total quantity of starch and protein digested can not be calculated; i.e., as has been noted previously, only the state of digestion of the material remaining in the stomach is known. However, minimum values for the quantity of both carbohydrate and protein digested can be calculated. In the case of the 1 hour digestion period with the finely divided meal, 50.5 per cent of the carbohydrate, or 15.2 grams, and 37.5 per cent of the nitrogen, or 6.08 grams as protein (0.98×6.2) remained in the stomach. Of these quantities, 2.98 grams of carbohydrate had been digested to reducing sugars (expressed as maltose) and 0.149 gram of protein had been digested to a soluble form. Thus it can be said that at least 9.9 per cent (2.98/30) of the carbohydrate and only 0.92 per cent (0.149/16.3) of the protein is digested to the prescribed end point when a meal of this nature is ingested by the average normal human subject.

It should not be concluded that, on the basis of these observations, the author is dismissing the stomach entirely as a site of protein hydrolysis and concluding that it is indispensable in the digestion of starch. The factor of safety in digestion is unquestionably great, and in man as in the dog (9) pancreatic amylase would be sufficiently abundant to hydrolyze any reasonable quantity of starch in the complete absence of ptyalin. It should be remembered, however, that disturbed nutrition is not the only way in which digestive inadequacies may be manifested.

The fact that only 2.5 per cent of the "nitrogen" was reduced to a soluble form in 1 hour does not, of course, mean that the remaining 97.5 per cent was completely undigested. In the hydrolysis of complex substances such as proteins and starches the earliest cleavage products are a mixture of complex and simple substances, with the former predominating. As

digestion proceeds there is an increasingly greater rate of formation of the ultimate hydrolytic products. Unquestionably a large fraction of the protein had been partially digested, although not to a tungstic acid soluble form. It should be noted, however, that in all cases the material recovered from the stomach contained appreciable quantities of readily recognizable meat, but no native starch as indicated by the iodine test.

On the basis of these results, there appears to be ample justification for the conclusion that, in the past, in considering the importance of the human stomach in digestion, the emphasis has generally been misplaced. It would appear that the importance of the stomach in the hydrolysis of starch, as a result of the action of ptyalin, has not been fully appreciated while its importance in the hydrolysis of protein has been over emphasized.

SUMMARY

Using young normal male adults as subjects, the relative importance of the human stomach in the digestion of protein and starch has been investigated. The experiment consisted of feeding the subjects a meal of known composition, removing it from the stomach at the end of a specified period of time, and determining the ratio of "digested" to "undigested" protein and starch in the material recovered from the stomach. The production of reducing sugars in the case of starch, and the production of nitrogenous material soluble in 2.5 per cent tungstic acid in the case of protein, were arbitrarily selected as end points of digestion.

In 4 experiments in which the test meal corresponded with what might be considered an "average" meal, samples for analysis were obtained by having the subjects empty their stomach as completely as possible by voluntary regurgitation at the end of either 0.5 or 1 hour. Under these circumstances it was found that, irrespective of the time that the meal remained in the stomach, approximately 40 per cent of the remaining carbohydrate was in the form of reducing sugars, while essentially none of the protein had been reduced to a form soluble in tungstic acid.

In 13 experiments in which a finely divided meal was used, the stomach was completely evacuated at the end of either 1 or 2 hours by means of an Ewald tube. Under these circumstances it was found that of the material remaining in the stomach at the end of 1 hour, an average of 19.6 per cent of the starch was in the form of reducing sugars while only 2.45 per cent of the nitrogen was soluble in tungstic acid. When the digestion period was extended to 2 hours, 9.5 per cent of the nitrogen remaining in the stomach was in a "digested" form.

In view of these observations it is felt that the conventional concept that the stomach plays an unimportant rôle in the digestion of starch and an important rôle in the digestion of protein, is open to revision.

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THE EFFECT OF HISTAMINASE ON THE GASTRIC SECRETORY RESPONSE TO HISTAMINE[1]

A. J. ATKINSON, A. C. IVY AND VIVIAN BASS

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

In a previous study we (1) found that histaminase given subcutaneously and slowly intravenously failed to influence the gastric secretory response to histamine and to a meal. Since then reports have appeared indicating that histaminase given orally or intramuscularly may possess therapeutic value in allergic conditions (2) and may depress the gastric secretory response to histamine and to a cold bath (3).

This work was undertaken to study the effect of histaminase, given intramuscularly to dogs and orally to human subjects, on the gastric secretory response to histamine. Such work was not included in our previous study because the negative results we obtained were theoretically predictable on the basis of a consideration of some of the *in vitro* observations of Best and McHenry (4), who for similar reasons have expressed doubt regarding the therapeutic promise of histaminase (5).

The histaminase powder used. The kidney histaminase used was obtained through the courtesy of the Winthrop Chemical Company. All lots were assayed by us a few days prior to their use. Fifteen milligrams of the powder were found to contain one unit of histaminase. We also made a preparation of histaminase from dog's intestinal mucosa; it contained one unit of histaminase in either 30 or 45 mgm. depending on the lot, and no histamine.

EXPERIMENTS AND RESULTS. *Heidenhain pouch dog.* After collecting the continuous secretion for three one-half-hour periods, 0.25 mgm. histamine dihydrochloride was injected subcutaneously and 10 minutes later the injection was repeated. The secretion was collected at 10 minute intervals for one hour, when the response to histamine had about disappeared. The total volume of secretion for one hour was measured. The histaminase was then injected intramuscularly and 25 or 30 minutes later histamine was injected as before. This time interval between the histaminase and histamine injections was chosen because it is one reported to be effective (3).

Eight units of kidney histaminase were injected in 7 experiments in a Heidenhain pouch dog on different days. The results are summarized in table 1. It is to be noted that on the average the secretion of acid was slightly reduced. Since the response of an animal to histamine varies from day to day and even to the same dose given 1.5 or 2 hours apart, it is necessary to test the significance of the differences observed by statistical methods. When the data are statistically analyzed, the difference in

TABLE 1

Showing the summarized results before and after histaminase given intramuscularly on the gastric secretory response to histamine (0.25 mgm.) in dogs

TYPE OF ANIMAL PREPARATIONS AND DOSE OF HISTAMINASE	NUMBER OF TESTS	VOLUME OUTPUT OF JUICE		OUTPUT OF HCl		REMARKS
		Before	After	Before	After	
	,	cc.	cc.	mgm.	mgm.	
Heidenhain pouch. 8 units of kidney histaminase	7	7.4 ±0.4*	6.9 ±0.6*	20.3 ±1.4	16.0 ±1.9	The difference in HCl output is 4.3 ± 2.3 and is not significant
Heidenhain pouch. 15 units intestinal histaminase	7	8.2 ±1.0	8.3 ±1.0	30.9 ±5	29.3 ±4	The lack of a difference is obvious
Total gastric pouch. Vagi cut. 30 units kidney histaminase	12	85.4 ±9.3	76.3 ±9.3	410 ±45	358 ±40	The difference in HCl output is 52 ± 50 and is not significant
Total gastric pouch. Vagi cut. 30 units inactivated kidney histaminase	8	80.2 ±7.4	67.7 ±8.6	406 ±40	324 ±45	C.R. of volume output is 1.29 and of acid output 1.06. The difference is insignificant

* Standard error of mean.

the volume and acid output before and after histaminase is found not to be significant.

Believing that a larger dose of histaminase might yield significant differences, 15 units of intestinal histaminase were injected in 7 experiments, the experimental procedure being the same as in the foregoing group of experiments. A significant difference was not observed. The results are summarized in table 1.

Total gastric pouch dog, with vagi cut. Thirty units of kidney histaminase were injected intramuscularly in 12 experiments. The experimental procedure and the dose of histamine were the same as in the experiments on

the Heidenhain pouch dogs, except the secretion was collected for seven instead of six 10 minute periods.

The volume and acid output appeared to be decreased after histaminase. It was decreased in 7 of the 12 experiments, increased in 3 and no change occurred in 2. When the differences were analyzed statistically, no significant change was found.

In view of these results it seemed unnecessary to conduct controls with *inactivated histaminase.* This was done, however, in 8 experiments to be certain that the histaminase powder contained no gastric secretory excitant other than histamine, the presence of which was ruled out by blood pressure assay. If it should contain such a secretory excitant, it would mask the effect of histaminase. This was not likely because the injection of the active histaminase powder was never observed definitely to stimulate. It is possible, however, that the tendency to diminished secretion that occurred in 7 of the 12 tests might be due to the injection of impurities present in the histaminase powder. The histaminase preparation was heated at 60°C. for 20 minutes to inactivate the enzyme.

The results of the intramuscular injection of inactivated histaminase are shown in table 1, and are almost identical with those obtained when the active preparation was administered.

Human subjects. Histaminase administered intraduodenally. Sixteen human subjects who were accustomed to swallowing a stomach tube were used. A double tube was swallowed, one of which passed about 10 inches into the duodenum and the other remained in the stomach. Alkaline fluid containing bile from the duodenal tube and acid fluid containing no bile from the gastric tube, showed that the tubes were in proper position. These criteria were required in all tests. After the double tube was in place the stomach was emptied of its residual fluid and the continuous secretion was collected for three 10 minute periods, saliva being expectorated. Then 0.5 mgm. of histamine dihydrochloride was injected subcutaneously and the secretion collected continuously, measured and titrated every 10 minutes for one hour and 10 minutes. Then, 100 units of kidney histaminase (1.5 grams) powder suspended in 30 cc. of 0.9 per cent NaCl solution were introduced into the duodenum through the duodenal tube, the tube being washed with 10 cc. of water. The duodenal tube was then clamped. The collection of gastric juice was continued. Twenty minutes after the introduction of the histaminase, 0.5 mgm. of histamine was injected and the gastric juice collected for 1 hour and 10 minutes.

In the first six subjects the test was performed only once. In the ten subjects who followed, the test was repeated about one week later.

The summarized results are shown in table 2. The histaminase obviously had no effect. The volume output of juice for each 10 minute period is shown in figure 1. A curve, curve *C*, showing the volume output

of juice of 20 subjects in 71 tests in response to 0.5 mgm. of histamine dihydrochloride is included in the figure.

Human subjects. Histamine administered orally for three days. Since histaminase is thought to be active when given orally in enteric coated

TABLE 2

Showing the response of 16 human subjects to histamine (0.5 mgm.) before and after the introduction of histaminase (100 cc.) into the duodenum

SUBJECT NUMBER	VOLUME OUTPUT FOR 1 HR. 10 MIN.				OUTPUT OF HCl FOR 1 HR. 10 MIN.			
	Before		After		Before		After	
	Average	Each test	Average	Each test	Average	Each test	Average	Each test
	cc.	cc.	cc.	cc.	mgm.	mgm.	mgm.	mgm.
1	88		120		170		227	
2	101		58		343		188	
3	90		91		157		178	
4	133		195		513		743	
5	156		146		706		694	
6	86		121		339		364	
7	99	101.5 97.0 184	102	109 95 125	443	489 396 731	415	497 332 322
8	172	160 100	154	183 102	672	611 256	462	602 257
9	119	138 130	115	127 162	308	360 578	306	354 624
10	137	144 97	155	148 95	613	647 380	560	495 353
11	99	101	92	90	362	344	358	362
12	116	108 129 70	103	113 94 221	426	388 465 164	368	398 337 472
13	69	68 131	197	172 128	150	136 437	549	525 381
14	120	108 292	115	102 329	408	379 1200	342	302 1347
15*	270	249 325	352	375 298	1026	852 1308	1139	931 1062
16*	276	227	265	233	1057	808	941	819
Average	133		149		481		490	

* The subjects had an active duodenal ulcer.

tablets, it is possible that the repeated administration of histaminase might be effective although a single administration might not be.

Accordingly, 8 subjects that responded quite consistently to histamine were given 150 units of histaminase daily for three days. Fifty units were taken in the form of enteric coated tablets (these were assayed and proved

to be active) one-half hour before meals. On the morning of the fourth day tablets containing 50 units were taken with some water one-half hour prior to the passage of the stomach tube. The histamine test was then performed as had been done prior to the three-day histaminase period.
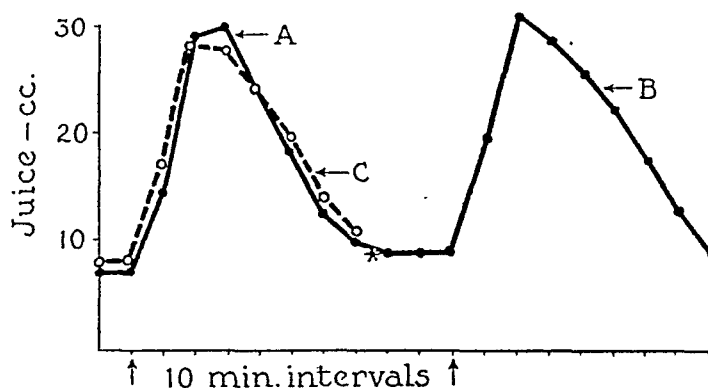
Fig. 1. Volume in cubic centimeters. Response to 0.5 mgm. histamine dihydrochloride.

——A = Before histaminase—26 tests on 16 subjects; 10 subjects tested twice.
——B = 25 minutes after histaminase.
-----C = Response of 20 subjects in 71 tests.
Output HCl in 1 hour, 10 minutes. A, 481 mgm.; B, 490.
Volume output, C = 140 cc.; HCl output 485 mgm.
* Histaminase, 100 units into duodenum.

TABLE 3

Showing the response of 8 human subjects to histamine before and after taking 150 units of histaminase in the form of enteric coated tablets for 3 days

SUBJECT NUMBER	VOLUME OUTPUT FOR 1 HR. 10 MIN.		OUTPUT OF HCl IN 1 HR. 10 MIN.	
	Before	After	Before	After
	cc.	cc.	mgm.	mgm.
1	172	180	672	752
2	119	121	308	334
3	137	166	613	521
4	99	90	362	220
5	99	116	443	521
6	179	208	631	669
7	107	117	416	447
8	80	78	134	136
Average.........	124	134	447	451

The results are shown in table 3. The histaminase had no effect on the response to histamine.

The data of five of these subjects on each of whom from 5 to 8 histamine (0.5 mgm.) tests had been performed were analyzed for variability to obtain some idea regarding the extent to which the response of the individual

varies from test to test. The subject manifesting the minimum variability secreted 93 cc. ± 5 cc. (S.E.) and 290 mgm. HCl ± 50 mgm. (S.E.). The subject manifesting the maximum variability secreted 166 cc. ± 14 and 602 mgm. HCl ± 87 mgm.

DISCUSSION. The failure of orally administered histaminase to decrease the gastric secretory response to histamine is not surprising in view of its inactivation by HCl-pepsin and by tryptic digestion (5) and of the fact that it has never been demonstrated that active enzymes are absorbed in significant amounts from the lumen of the intestine (6). Neither is it surprising that intramuscularly administered histaminase has no effect on the gastric secretory response to histamine, since we found previously that the continuous intravenous injection of histaminase during the absorption of histamine from subcutaneous tissue is ineffective (1).

Attention should be called to the fact that the response of the stomach to a standard dose of histamine is not constant. Hence, when the effect of a substance on gastric secretion is being studied, the significance of differences between averages should be examined by the statistical method.

Therapeutic studies of histaminase action to date are characterized by a failure to use inactivated histaminase as a control. Such a control would seem to be essential, particularly in view of the fact that all preparations of histaminase are very impure.

CONCLUSIONS

1. From 8 to 15 units of histaminase injected intramuscularly in a Heidenhain pouch dog and 30 units similarly injected into a dog with a pouch of the entire stomach failed to decrease the gastric secretory response to 0.5 mgm. of histamine dihydrochloride.

2. One hundred units of histaminase introduced into the duodenum 20 minutes before the injection of 0.5 mgm. of histamine failed to decrease the gastric secretory response in a group of 16 human subjects.

3. The administration of 50 units of histaminase in the form of enteric coated tablets three times daily for three days to 8 human subjects had no effect on their gastric secretory response to 0.5 mgm. of histamine.

In this and a preceding study we have been unable to demonstrate that histaminase given by any route has a significant inhibitory effect on the gastric secretory response to 0.5 mgm. of histamine. ,

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THE EFFECTS OF DIRECT CURRENTS UPON THE ELECTRICAL EXCITABILITY OF NERVE

A. ROSENBLUETH

From the Department of Physiology in the Harvard Medical School

Previous observations made in this laboratory (Reboul and Rosenblueth, 1939; Rosenblueth, Reboul and Grass, 1940) upon the action of alternating currents on mammalian nerves showed several effects which could not be accounted for satisfactorily on the basis of the standard knowledge of the action of direct currents (d.c.). The assumption that alternating currents might exert a qualitatively specific influence was unlikely. It appeared more probable that the understanding of the effects of d.c. was incomplete or faulty.

The present study deals with the changes of electrical excitability of nerves during or after the passage of d.c.

METHOD. The animals used were cats, either under dial anesthesia (Ciba, 0.75 cc. per kgm. intraperitoneally) or decapitated 1 to 4 hours previously under ether. The nerve studied was mainly the motor component of the popliteal, the contractions of the muscles attached to the Achilles tendon serving as indicators of the corresponding nerve impulses.

The sciatic was cut and 6 to 10 chlorided silver-wire electrodes were applied to the popliteal. The electrodes were insulated by rubber from the neighboring tissues. The popliteal was separated from the peroneal in three stretches of about 15 mm. for the insertion of the electrodes. Care was taken in making this dissection to preserve the blood supply of the nerves as intact as possible. Observations, in which certain drugs known to affect nerves have been injected intravenously after a similar preparation, have shown that such nerves do have an adequate blood supply.

The legs were fixed by drills inserted into the tibiae. The Achilles tendon was freed and attached to the short arm of a myograph. The muscles pulled against strong rubber bands. Contractions appear in the records as upward excursions of the tracing.

In some experiments the peroneal nerve was dissected from hip to knee and excised for study in a moist chamber. The electrodes were usually thin calomel half-cells making a contact with the nerves through agar-Ringer bridges and wicks moist with Ringer. The spike potential of the

A fibers served in these cases as indicator of the nerve impulses. The potentials were recorded, after suitable amplification, from a cathode-ray oscillograph.

The d.c. was obtained from batteries, the intensity being regulated by a potentiometer. Resistances of 25,000 to 125,000 ω were usually placed in series with the nerve, in order to prevent possible spurious shifts of the stimulated points when the test shocks were delivered at either of the d.c. poles. The e.m.f. and intensity were read during the passage of the current from a voltmeter and an ammeter, respectively. The applications of d.c. lasted from 1 to 15 seconds.

In some of the classical studies on electrotonus stress was laid on the desirability of using only one direction of d.c., "ascending" away from the muscle or "descending" toward the muscle in any given experiment. That practice was not followed in this study, since it was found that the effects of a given first application could be duplicated accurately by a similar treatment following one or more applications of d.c. in the reversed direction. The only condition necessary for such accurate reduplication was a reasonable wait (1 to 5 min.) between the observations. Indeed, it was found that systematic reversal of the d.c. led to more consistent results than those obtained with repeated unidirectional treatment. In the excised nerves a slowly progressive change of effects was the rule, due probably to the absence of circulation. In the circulated nerves the early results could be reasonably reproduced even after several hours of observation as long as the intensity of the currents used was relatively moderate.

The test shocks were condenser discharges of various capacities and intensities. When these shocks were repeated regularly the frequency was controlled by a thyratron. The use of condenser discharges, instead of induction shocks, allowed the study of the polar and interpolar regions of the nerves without the establishment of additional poles for the d.c. The study of the excitability at any given point in the nerve was made with both ascending and descending test shocks. That is, while the stimulating cathode for the test shock remained fixed, the anode was made first proximal, then distal to the cathode with respect to the muscle, or *vice versa*.

RESULTS. A. *Changes of excitability at the d.c. poles.* Increased excitability at the cathode and decreased excitability at the anode throughout the passage of even quite prolonged (several minutes) d.c. are too well known to deserve any further comment.

The decrease of excitability at the cathode found by Werigo (1883) has likewise been extensively studied. This decrease is produced either by the brief application of strong currents or by the prolonged delivery of moderate currents. In either case the intensification or prolongation of the current exaggerates further the decrease of excitability. There is,

however, another type of cathodal depression, produced by very weak currents. Intensification of the current leads in these cases to the appearance of the usual increase of excitability.

In figure 1 is illustrated a typical example of the second type of cathodal depression. As shown in the records from A to D, progressive intensification of the current causes first an increase of the depression but leads later to the appearance of increased, instead of decreased excitability. Record E shows that long interelectrodal distances are unfavorable to the appearance of depression. And record F illustrates the existence of a
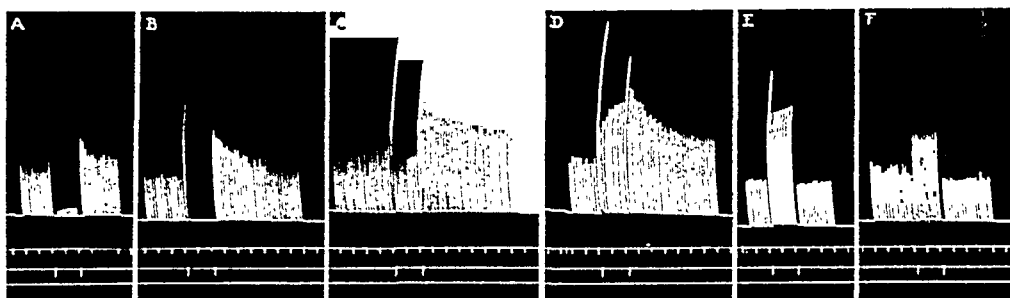
Fig. 1. The second type of cathodal depression. Cat under dial. Muscular responses to submaximal stimulation of the popliteal nerve. D.c. was applied in a descending direction, as shown by the signals. The test stimuli were applied in the ascending direction, the cathode being common to that of the d.c.—i.e., the order of the electrodes beginning centrally was: first, the d.c. anode, 6 mm. away the common cathode, and 10 mm. below that the anode for the test stimuli. A resistance of 25,000 ω was in series with the battery. The condenser discharges had a capacity of 0.01 μF and discharged through the nerve and a series resistance of 18,000 ω. The voltages of d.c. applied to the nerve were: A, 0.05; B, 0.25; C, 0.75; and D, 1.0 v.

In E the interelectrodal distance for the d.c. was increased from 6 to 12 mm. by moving the anode. The voltage of d.c. was 0.25 (cf. B).

In F the d.c. was delivered as in B. The cathode of the test stimulus was moved, however, 10 mm. peripherally to the d.c. cathode, and the anode of the test shock was moved correspondingly.

In this and the following kymograph records the time signal denotes 5-sec. intervals.

region of increased excitability outside the cathode, although the excitability at the cathode itself is depressed (B).

An increase of excitability at the anode can be easily demonstrated by several procedures. In figure 2 is illustrated a typical series of observations using condenser discharges as the test stimuli. While weak currents cause anodal depression (A), strong voltages produce increasingly augmented responses (B to D). The results are seen whether the test stimuli reach the anode of the d.c. in an ascending (B) or a descending (E) direction. While the excitability is increased at the anode (B and E) it is decreased at a point a few millimeters outside (F).

Another method used for the study of the polar changes of excitability

was the following (fig. 3). Two sources of d.c. were connected in parallel to the same pair of electrodes on the nerve. A given high resistance (10,000 to 100,000 ω) was placed in series with each of the d.c. sources.
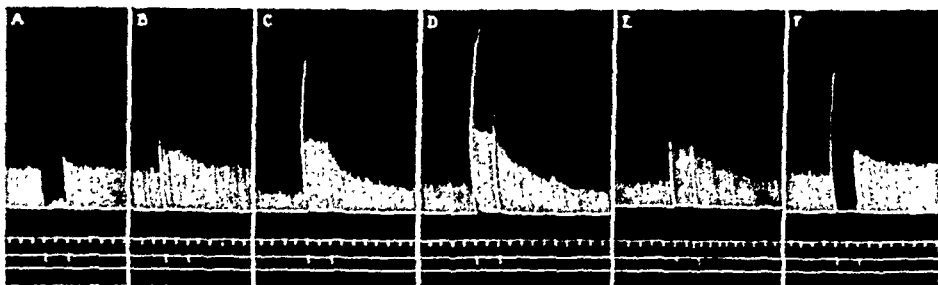
Fig. 2. Anodal increase of excitability. The procedure followed for the tests was similar to that in figure 1. The order of the electrodes from central to peripheral points of the nerve was: d.c. cathode; 19 mm. further, common electrode for the d.c. anode and the test stimulus cathode; 7 mm. further, stimulus anode. The voltages of d.c. were: A, 0.02; B, 0.05; C, 0.13; D, 0.25.

In E the anode of the test stimuli was moved to a point midway between the d.c. electrodes. The stimuli were now descending instead of ascending. Voltage, 0.05.

In F the stimulus cathode was moved to a point 7 mm. peripheral to the d.c. anode.

Fig. 3.

Fig. 4.
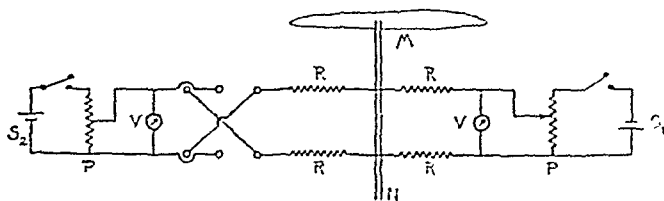
Fig. 3. Diagram of the arrangement for testing the electric excitability by brief d.c. pulses through the same electrodes used for continuous application of d.c. from another source. The letters have the following meaning: M, muscle; N, nerve; R, 10,000 to 100,000 ω; V, voltmeter; P, potentiometer; S, source of d.c.
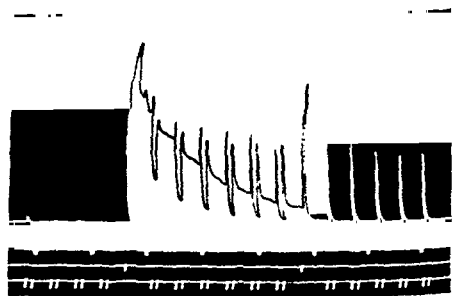
Fig. 4. Increased excitability at the anode during the passage of d.c. Decapitate cat. The experimental set-up was as shown in figure 3. The voltages were measured across the resistances in series with the nerve; the drop of potential in the nerve was much less. Interelectrodal distance: 15 mm. Just threshold (1 v.) brief d.c. pulses were applied from one of the sources in the descending direction, as shown by the pairs of lower signals. The middle signal indicates the period of application of d.c. (12 v.) from the other source in the ascending direction.

The same value was used on the two circuits so that the resistance of the two loops was equal. A simple calculation shows that the drops of potential across the branch common to the two loops (that is, across the nerve) sum algebraically. In other words, the drop of potential impressed on the

nerve by one of the sources will be added to or subtracted from any drop impressed by the other source.

The experimental procedure was to apply regularly brief (0.5 to 2 sec.) descending pulses from one of the sources before, during and after the delivery of an ascending or descending prolonged (5 to 15 sec.) current from the other source. The advantages of the method are first, that, as pointed out above, the currents across the nerve are independent; second, that the responses to the make or break of the brief test pulses can be readily distinguished.

By the use of this method all the polar changes described thus far could be readily confirmed. In figure 4 is shown a typical record exhibiting increase of excitability at the anode. The preliminary test d.c. pulses caused only slight responses at the make. The prolonged ascending d.c. from the other source resulted in a sustained tetanic response. Interrupting this response are seen the now large brief tetani caused by the make of the test pulses. Such make is equivalent to a sudden weakening of the d.c. applied, since the two currents flowed in opposite directions. The drop in the sustained tetanus during the closure of the test is due to this relative weakening. The opening of the tests is equivalent to a sudden intensification of the continuous current, and results in turn in a strong tetanic response. A post-anodal increase of excitability is shown by the large responses to the test stimuli after the prolonged d.c. was interrupted.

An independence of the polar effects was indicated by the fact that when d.c. of a certain intensity increased the excitability at one pole it could cause either an increase or a decrease at the other pole. This independence is illustrated in figure 5.

In some of the experiments the polar changes of excitability were studied, using electrodes shielded by glass (Sherrington electrodes) for the application of d.c. The purpose of these observations was to isolate the tested region of the nerve from surrounding tissues. Although higher voltages were necessary for the appearance of some of the changes described above, they could all be confirmed. The relatively greater difficulty found for the demonstration of anodal enhancement or cathodal depression of the type illustrated in figure 1 is attributed both to impairment of the blood supply of the nerve and to the proximity of the region tested to the cut end.

The importance of the blood supply was seen in controls in which this supply was abolished, leaving the nerve *in situ* and placing the electrodes as usual. In such nerves the effects described above were difficult to obtain or did not appear at all. The importance of testing regions of the nerve at some distance from the cut end was readily demonstrated by applying similar tests first near and then far from the cut. In the first case only Pflüger's classical effects could be shown.

B. *Juxtapolar changes.* A quantitative description is given below

(section G) of the changes of excitability throughout all the regions of the nerve, polar, interpolar and extrapolar, produced by the applications of d.c. The purpose of this section is to emphasize that the effects at the poles may be quite different in sign and magnitude from the changes in regions of the nerve distant only a few millimeters away. These differences are especially noticeable when the currents applied lead to an increase of excitability at the anode or a decrease at the cathode (see figs. 1 and 2).

C. *Distant extrapolar changes.* Wedensky (1920) reported that when the tests of excitability were carried out far enough away from the d.c. poles the sign of the change was reversed. Thus, if an ascending current resulted in a decreased excitability of the regions of the nerve within 2 or
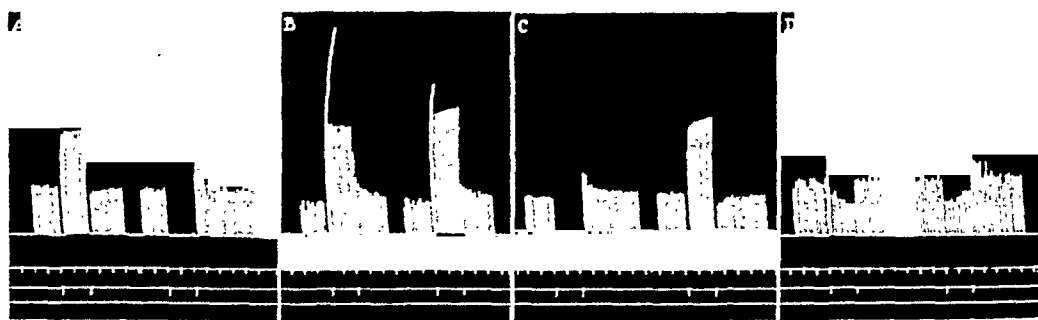
Fig. 5. Independence of the polar effects. All the tests were made using the pole of the d.c. proximal to the muscle as the cathode for ascending test shocks. The records are arranged in pairs, showing the effects at the same point, first of an ascending, then of a descending d.c.

A. Anodal increase and cathodal decrease. Voltage of d.c. 0.05. Interelectrodal distance: 6 mm.

B. Anodal and cathodal increases. Voltage: 0.1. Interelectrodal distance 6 mm.

C. Anodal decrease and cathodal increase. Voltage: 0.1. Interelectrodal distance 15 mm.

D. Anodal and cathodal decreases. Voltage: 0.1. Interelectrodal distance: 12 mm. The test capacity was smaller (0.001 μF) than the one used for the other tests (0.01 μF).

3 cm. from the anode, the excitability of regions distant 4 to 6 cm. from that pole was increased. Conversely, the cathodal extrapolar segment showed first an increase, then a decrease of excitability as the points tested were further from the pole. Wedensky spoke of the regions of the nerve where this reversal was found as the peri-electrotonic zone.

Wedensky's report was easily confirmed. The reversal of the excitability changes in the extrapolar regions was seen even with weak currents. For a given distance between the d.c. electrodes the stronger the current, the closer to the pole was the point at which reversal took place.

D. *The measurement of the changes of excitability.* A change of excitability could be due to a modification of either the intensity or the time parameter or of both parameters of the strength-duration curves of the

nerve fibers. The qualitative determinations illustrated in figures 1, 2 and 5 merely indicate that the nerve as a whole has become hyper- or hypo-excitable in a certain region, since more or less fibers, respectively, are activated by the invariant test stimulus.

In order to gain a more accurate insight of the changes produced, volt-age-capacity curves were constructed as follows. The end-point was a response of constant small magnitude (usually about 20 per cent of the maximal twitch). This insured the test of a constant number of fibers. The d.c. and the test-stimulating electrodes were maintained in a fixed position. The normal voltage-capacity curve (without d.c.) was first determined in the usual manner. The normal intensity value for a given capacity was then re-ascertained. A certain fixed voltage of d.c. was then applied and maintained until the voltage of the test stimulus for the con-stant response was determined (3 to 5 sec.). The d.c. was discontinued. One to 5 minutes later the normal value was checked, the d.c. was re-applied but in the reversed polarity, and the new threshold was determined as before. The procedure was then repeated with another capacity until the whole range of the curves had been explored. The repeated test of the normal values first obtained insured that no progressive change was present which would vitiate the results. The determinations were made first with decreasing and then with interpolated increasing capacities. The three curves (normal, anodal and cathodal) were plotted in double logarithmic scales and analyzed for the values of the voltage (r) and time (k) para-meters, according to Hill's (1936b) method.

The results were as follows. The outstanding change produced by d.c. was in the voltage parameter (rheobase), but the time parameter was in-variably likewise affected. As a rule, a decrease of rheobase was attended by an increase of k and *vice versa* (fig. 6A). An increase of the time para-meter denotes decreased excitability. A decrease of rheobase leads, on the contrary, to greater excitability. Since both changes usually took place simultaneously, it is clear that the net modification of excitability depended on which of the two changes was more prominent. As already stated, the rule was for the change in rheobase to determine the final reactivity for all durations (fig. 6A). It was possible, however, to obtain conditions in which the electrotonic curve crossed the normal curve (fig. 6B). In such cases, if the test was made with a brief shock the nerve appeared hypoexcitable during the passage of d.c., whereas it appeared hyperexcitable if the test shock was of relatively long duration.

Since the changes of excitability produced by d.c. involve both the voltage and the time parameters it is not possible to measure such changes in a simple direct manner. The predominant change, however, is that in the rheobase. It was decided, therefore, that an approximate measure-ment could be reached by determining the voltage threshold for a response

of fixed magnitude with a relatively large capacity. The ratio of the normal value of this threshold to the value obtained during an experimental procedure multiplied by 100 expresses the percentile modification of excitability.
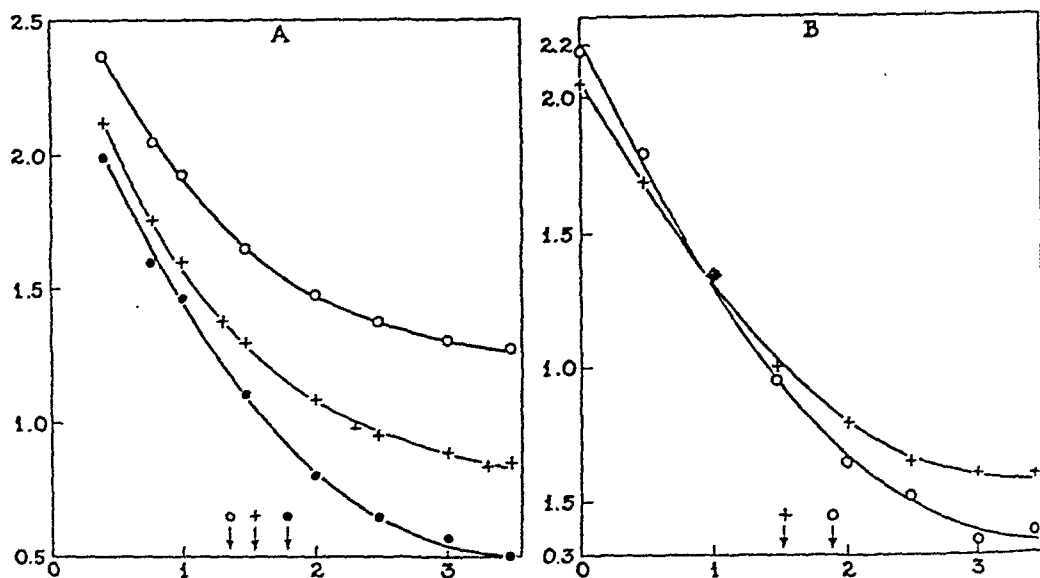
Fig. 6. Voltage-capacity curves during the passage of d.c. The curves are plotted in logarithmic scales. Abscissae: $\log_{10}$ capacity; the unit is 0.001 μF. Ordinates: $\log_{10}$ voltage; unit, 0.1 v. The scale for the ordinates is twice that of the abscissae. The arrows indicate the value of Hill's constant k.

A. The interelectrodal distance for the d.c. was 16 mm. The cathode of the test stimuli was 6 mm. below the peripheral d.c. pole. Resistances of $9{,}000\omega$ and $1{,}800\omega$ were placed in series and in shunt, respectively, in the condenser circuit. Crosses: normal curve, without d.c.; some of the points were taken before, some after the applications of d.c. for the construction of the other curves. Circles: curve obtained during applications of ascending d.c. (0.5 v.; anodal polarization). Dots: curve obtained during applications of descending d.c. (same voltage; cathodal polarization).

B. Interelectrodal distance for the d.c.; 10 mm. Test cathode at the peripheral pole of the d.c. Resistances of $10{,}000\omega$ were in series and in shunt with the nerve in the condenser circuit. Crosses: normal curve. Circles: during applications of ascending d.c. (0.5 v.; anodal polarization). The curve obtained during cathodal polarization is omitted in order not to obscure the crossing of the normal and anodal curves.

E. *The influence of voltage.* Changes in the intensity of the d.c. applied resulted not only in quantitative variations of the effects observed, but could result also, as already pointed out (figs. 1 and 2), in a reversal of the sign of a given effect. Using the method just described for the measurement of the electrical excitability, curves were traced correlating the sign and magnitude of the changes with the voltage of the currents applied.

No uniform description of these curves can be given because the results

varied with the interelectrodal distance for the d.c. and the region of the nerve tested. The extreme changes of excitability found were 350 and 20 per cent of the normal. The curves often crossed the line of normal excitability. Thus, low voltages could produce a decrease and higher voltages an increase of excitability at the same point of the nerve and with a constant polarity of d.c. (see figs. 1 and 2).

Qualitatively the following statements summarize the influence of voltage on the variations of excitability. Weak currents favor the appearance of a decrease at the anode and cathode. Stronger currents promote increased excitability at both the anode and the cathode. With even stronger currents the increase at the anode grows, whereas the increase at the cathode declines or is replaced by a decrease. All the intrapolar and extrapolar changes become more prominent with increasing voltage.

F. *The influence of the interelectrodal distance.* The distance between the d.c. electrodes was of importance in the results obtained. With equal voltage drop across the electrodes or equal intensity of current flowing through the nerve, variations in the interelectrodal distance resulted not only in quantitative differences of effects (Pflüger, 1859) but also could result in a reversal of sign of the change of excitability elicited at a given point.

The most marked effect seen in this respect was on the appearance of an increase of excitability at the anode. Thus, 2 to 8 times stronger voltages were necessary to elicit an anodal increase if the interelectrodal distance was doubled, for instance, from 6 to 12 mm., by moving the cathode without disturbing the anode. A relatively short span of the d.c. poles was also found to favor the appearance of cathodal depression.

Not only the distance between the d.c. poles, but also the length of nerve included between the two electrodes used for the delivery of the test shocks was of importance for the results recorded. Thus, anodal enhancement could be obtained with weaker d.c. if that length was short than when the stimulating electrodes were widely separated. Proper controls showed that the influence of the length in question was more significant than that corresponding to the direction, similar or opposite, of the test current with respect to the d.c.

G. *The distribution of effects along the nerve.* The mapping of the changes of excitability at different regions of the nerve produced by a given application of d.c. was effected as follows. Referring to the 10 electrodes on the peroneal nerve by numbers, *1* to *10*, two of them were selected for the applications of d.c. with constant voltage, e.g., *3* and *5*. A given test capacity was then discharged in the descending direction from *1* to *2*, and the voltage necessary for a fixed response was determined. This value, obtained without d.c., gave the normal threshold for that position of the test. The d.c. was then applied with the ascending polarity and the thresh-

hold was redetermined within 3 to 5 sec. After a pause, the normal value was checked, d.c. was applied in the reversed direction, and the new threshold was determined. The changes of excitability at that point could then be calculated as explained in section D. The test was then moved systematically to the next two electrodes on the nerve and the procedure was repeated. The measurements were carried out until electrodes *9* and *10* were reached and then the series was repeated with ascending tests in the reversed direction: *10, 9; 9, 8;* etc.

The values obtained were attributed to the point of the nerve where the cathode of the test was placed. There were, therefore, two values for each of the electrodes *2* to *9*, and one for the electrodes *1* and *10*, for each of the directions of the d.c. The curves were plotted with the distances between the points tested and the d.c. poles as abscissae, and the corresponding changes of excitability as ordinates.

In order to make the changes seen with one polarity of d.c. comparable with those obtained with the reversed polarity, the plot was first made of all the values obtained with the ascending current. The values for the descending current were then plotted on the same graph, using the points already marked for the anode and cathode as references for the abscissae. In other words, whether the observations were made with an ascending or a descending current, the changes at the cathode, or those at a given distance from the cathode in the cathodal extrapolar region, etc., were plotted at the corresponding point on the graph. With this procedure at several of the points in the abscissae there were as many as four values for the ordinates.

The results were quite consistent in any series of determinations. Occasionally, some discrepancies were seen when comparing the values obtained with the ascending or descending d.c., or with the ascending or descending test stimulus, for a given point on the nerve. These discrepancies were more frequent for the d.c. poles than for any other region of the nerve. But even when such discrepancies were present a repetition of any of the determinations at the end of the series agreed with the values obtained previously.

As shown in the preceding sections, at least four independent variables are involved in the changes of excitability produced by d.c. at a given point of the nerve: d.c. interelectrodal distance, d.c. voltage, distance of the point from the d.c. poles, and duration (capacity) of the test stimulus. Even if the last factor is dismissed from consideration for the reasons given in section D, only a family of surfaces could give a complete graphical description of those changes. Rather than attempting to project such a family of surfaces into a two-dimensional drawing, some experimental curves are illustrated in figure 7.

Curve A is typical for a moderate interelectrodal distance and intensity

of d.c. The curve is fairly similar to Pflüger's (1859) classical law, but a slight extrapolar reversal is clear. With slightly shorter interelectrodal distances the maximum and minimum of the excitability changes were displaced to the extrapolar regions. Curve B shows the presence of anodal enhancement. In many instances the increase at the anode was only relative, that is, the excitability was depressed, but less that in neighboring inter- and extrapolar regions. In curve C both anodal enhancement and cathodal depression are present. Cathodal depression
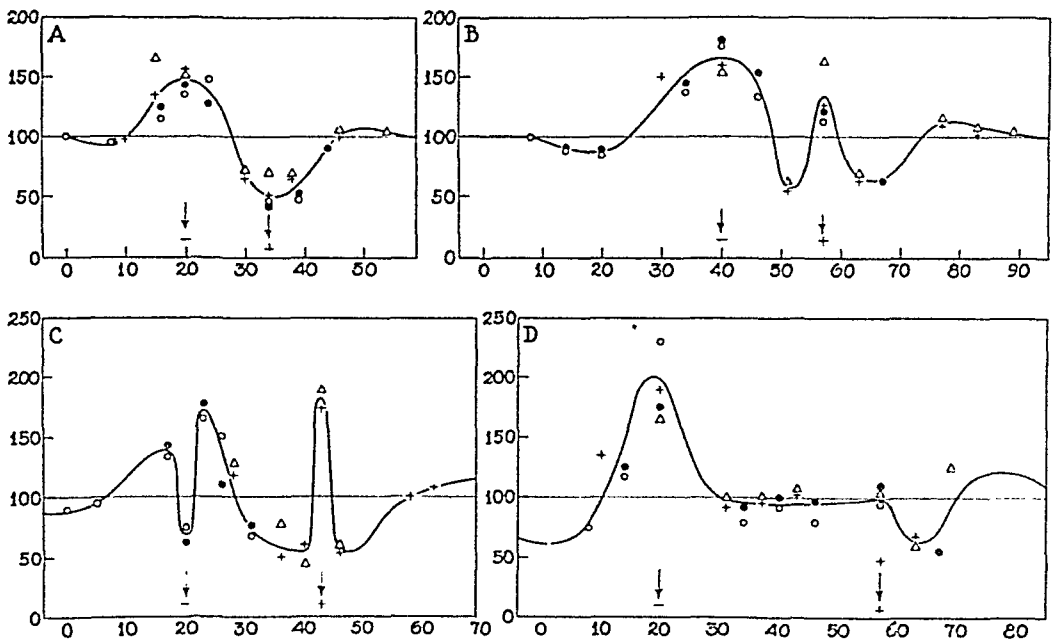
Fig. 7. Different types of curves describing the distribution of excitability changes in different regions of a nerve upon applications of d.c. Ordinates: per cent excitability (referred to the normal). Abscissae: distances in millimeters along the nerve. The arrows and signs indicate the position of the d.c. electrodes on the nerve. Crosses and triangles: points determined for ascending d.c. with ascending and descending stimuli, respectively. Circles and dots: values for descending d.c. with ascending and descending stimuli, respectively. Further explanation of the method in text. The voltages of d.c. were 0.3, 0.5, 0.5 and 0.8 for A to D, respectively.

was in turn sometimes only relative—i.e., less increase than in neighboring points. Curve D is typical of long interelectrodal distances, in showing practically normal excitability in most of the interpolar region.

H. *The lack of polarity in motor fibers.* By polarity in a nerve fiber is meant any systematic change along the longitudinal axis such that the more central region of the fiber would exhibit properties differing quantitatively from those of more peripheral regions. An absence of polarity with regard to electrical excitability was shown by two sets of observations. First, the threshold of the nerves tested with either ascending or descending

condenser discharges of a fixed capacity (0.001 to $1\mu F$) varied only in a random and slight degree for points of the nerve from 3 to 8 cm. below the cut central end, provided the interelectrodal distance was approximately constant. The random variations can be explained by slight differences in the contacts made by the corresponding electrodes with the nerve. Second, the determinations of changes of excitability made with ascending currents agreed quantitatively, as a rule, with those made with descending currents (fig. 7).
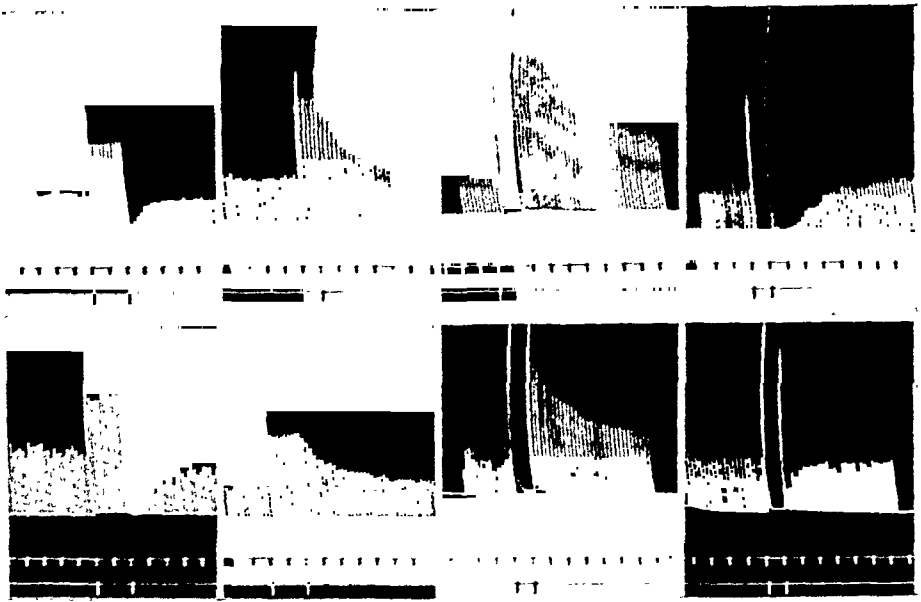
Fig. 8. Independence of the after-effects of d.c. from the changes produced during the passage of the current. All the tests were made with the cathode of the test stimulus common to the peripheral d.c. pole. The stimuli were ascending, that is, the anode was further peripherally. The upper row of records is for ascending d.c.—i.e., tests of the anodally polarized point. The lower row is for descending currents—i.e., tests of cathodally polarized point. None of the currents used was strong enough to produce a block of nerve impulses.

It is obvious that if axons are not polarized, but uniform, the distinction between ascending and descending currents is unimportant except when blocking effects are developed. For this reason the direction of the current in the nerve was not qualified in sections A to F.

I. *The after-effects of d.c.* The results described thus far have dealt with effects encountered during the passage of d.c. Because there is no reason to assume that the after-effects of a given application of current differ fundamentally from the changes encountered during the application, no quantitative study of these after-effects was attempted. Some interesting features of the numerous qualitative observations made are the following.

The after-effects at a given point may or may not have the same sign as the change produced during the passage of d.c. (fig. 8). In some instances, especially with relatively strong voltages of d.c., first an increase and later a decrease of excitability was observed, or *vice versa*.

Post-cathodal depression is apt to occur after weak currents, especially if the interelectrodal distance is long. Strong voltages, on the other hand, favor the appearance of a post-cathodal increase of excitability (Pflüger, 1859). Post-anodal depression is likewise more prominent after strong than after weak currents. With a constant interelectrodal distance greater voltages were usually necessary to convert the post-anodal increase of weak currents into a post-anodal depression than those sufficient for the transition from a post-cathodal depression to an increase.

J. Observations on excised nerves. As mentioned under Method, the indicator of nerve activation was in these cases the spike potential of the A fibers. The d.c. was delivered through non-polarizable calomel electrodes. The results differed from those in the circulated nerves as follows.

Anodal enhancement of excitability was only seen with quite short (3 to 6 mm.) interelectrodal distances. A further important precaution for the appearance of this effect was to test a region of the nerve well away (at least 3 cm.) from the cut ends. With this precaution very weak voltages led to classical depression at the anode during the passage of d.c. Slight intensification of the current (0.02 to 0.04 v. across nerve) resulted in anodal enhancement. Further intensification (e.g., 0.1 v.) caused again a depression. Figure 9 illustrates typical observations.

Reversal of the extrapolar cathodal effects—i.e., transition from increase to decrease of excitability—was readily seen. The similar reversal in the anodal extrapolar region (p. 62) was not seen clearly in any instance.

DISCUSSION. A comparison of the present results with those obtained by Pflüger (1859) shows that Pflüger's observations were accurate but incomplete. His interpolations and extrapolations were, therefore, inadequate. His observations would fit the curves in figure 7, yet some of these curves differ considerably from the classical laws reproduced in all text-books. Instead of the one "indifferent point" separating the anelectrotonic, depressed zone, from the catelectrotonic, enhanced region of the nerve, there may be as many as 7 "indifferent points" during an application of d.c. (fig. 7C).

Cathodal depression (Werigo, 1883) and anodal increase of excitability (for references see Mares, 1913; Thörner, 1923; and Ebbecke, 1933) have been frequently reported. But the appearance of these effects has been usually considered indicative of abnormality of the nerves. Blair and Erlanger (1936) describe a cathodal depression in normal nerves treated with weak currents, but this depression is only relative; instead of an absolute decrease of excitability it appears in their observations as a reduction of the original increase produced by the current.

The cathodal depression and anodal increase shown in figures 1, 2, 4, 5 and 8 cannot be attributed to abnormal conditions. The effects appeared with weak currents and the nerves were kept as fresh and intact as possible. Indeed, as emphasized in sections A and J, the phenomena were less clear or could not be obtained in abnormal nerves, nerves deprived of their circulation or excised.

Evidence of anodal enhancement can be shown by a procedure different from those employed here (Rosenblueth, 1941a). Impulses traveling over some of the fibers in a nerve may stimulate adjacent fibers at the region of the anode of an applied d.c. The phenomenon is only seen in fresh nerves. Again, an increase of excitability at the anode during the passage of d.c. sufficient to result in excitation of the nerve is best seen in normal nerves (Rosenblueth, 1941b).

In an effort to bring together all the effects of d.c. upon nerve in a relatively simple schema, Ebbecke (1933) suggested that under the influence of increasingly stronger cathodal polarization a nerve's excitability is first increased to an optimum but later depressed until total failure to conduct takes place (see Wedensky, 1903). The anodal influence would be opposite in character to the cathodal effect. Thus, depending on the state of the nerve at the time of the test either cathodal or anodal polarization could increase or decrease excitability by causing the nerve to approach or depart from the condition of optimal excitability.

There are many observations not explained by this simplified schema. According to it, whenever weak cathodal or anodal polarization causes a decrease of excitability, intensification of the current should lead to greater depression, because the state of the nerve would be moved further away from the condition of optimal excitability. Intensification may lead, however, from depression to enhancement (figs. 1 and 2). Furthermore, according to the schema, weak currents should invariably result in opposite effects at the anode and cathode. This corollary is not supported by the observations in figures 5 and 9.

The following factors were found to influence the effects of d.c.: a, distance between the d.c. electrodes (figs. 1 and 2, section F); b, voltage (figs. 1, 2 and 9; section E); c, distance of points tested to the d.c. poles (figs. 1, 2 and 7; section G); d, duration of the test shocks (fig. 6; section D); e, interelectrodal distance for the test stimuli (section F). Although the last two variables are less important than the others for certain purposes, it is clear that no simple schema will do justice to the facts. Ebbecke's hypothesis considers only the influence of voltage. It is a 2-dimensional representation, whereas at least a 4-dimensional description is required by the data.

The term "accommodation" was introduced by Nernst (1908) to describe a rise in the threshold of nerve when a steady potential is applied

for some time. It is obvious from this definition that the concept of accommodation and the study of electrotonic changes of excitability are intimately related.

In developing his comprehensive theory of electrical excitability of nerve, Hill (1936a) dismissed electrotonic phenomena as secondary, on the basis of observations showing a decrease or even an inversion of the "usual" electrotonic effects. The fact that electrotonic changes are contingent upon the experimental procedure is not an adequate argument for considering them as secondary in the problem of excitation. Operationally the modifications of excitability produced by d.c. are inseparable from some of the facts recognized by Hill as of primary importance, e.g., the facts which lead to the concept of accommodation.
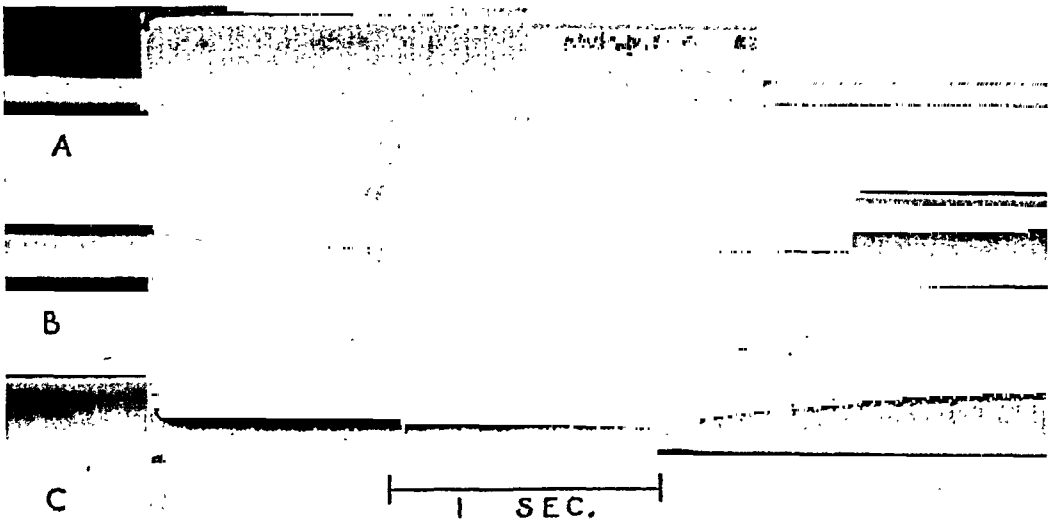
Fig. 9. Increased excitability at the anode during the passage of d.c. Excised peroneal nerve. Monophasic records of the spike potentials of A fibers. Submaximal test stimuli applied continuously at the rate of 120 per sec. The sudden changes in the records correspond to the closure and opening of the d.c. The distance between the d.c. electrodes was 5 mm. The anode of the d.c. coincided with the cathode of the test stimuli. Voltages of d.c.: A, 0.04; B, 0.1; C, 0.2.

Hill defines "normal accommodation" as a change in threshold such that when a nerve is at equilibrium with a given current the additional current required to stimulate is the same as it was originally, before any application of d.c. This equilibrium would be approximately attained within a very brief period after the application of d.c. As a simplification that definition may be useful. But the term "normal" is unsatisfactory. It is only very exceptionally, if ever, that this type of accommodation is seen experimentally. In optimum normal conditions, that is, in unanesthetized, circulated nerves *in situ*, applications of d.c. result in changes of excitability which persist for minutes not only at the poles but also at

points up to 6 to 8 cm. away from the poles.   Such applications may cause prolonged tetanic discharges if the voltage is adequate (Rosenblueth, 1941*b*).   A general definition of accommodation should include all the electrotonic changes, not only some selected instances.   With the data available there is no reason for separating the electrotonic phenomena from the phenomena of excitation.

The classical approach to the problem of the action of electricity upon nerve is to assume that the changes of excitability produced are direct consequences of the changes of polarization caused by the current: depolarization at the cathode and increased polarization at the anode.   This assumption is inadequate to cope with the complex effects encountered. It appears more likely that the action of the current is not direct, but that intermediate physico-chemical steps may play a rôle.   Such hypothetical intermediate processes would not necessarily be opposite at the anode and cathode.

The data (figs. 1, 2, 4 and 9) suggest that both the cathodal and the anodal influences include a factor for increasing and another for decreasing the excitability of nerve.   The preponderance of one or the other of the two factors in a given region of the nerve is determined by the experimental conditions.   An independence of the several influences is supported by the lack of uniformity seen when comparing the anodal or cathodal effects (fig. 5) or after-effects (fig. 8).   An interaction of the anodal and cathodal actions is seen, on the other hand, in the asymmetrical distribution of the changes at different points in the interpolar region of the nerve (fig. 7).

SUMMARY

The changes of electrical excitability produced by application of direct current (d.c.) were studied in cat's myelinated nerves, either circulated or excised.

There may be an increase or a decrease of excitability at the anode or the cathode during or after the passage of d.c. (figs. 1, 2, 4, 5, 8 and 9).

The following factors influence the results obtained: *a*, distance between the d.c. electrodes (figs. 1 and 2, section F); *b*, voltage of the d.c. (figs. 1, 2 and 9; section E); *c*, distance of the points tested to the d.c. poles (figs. 1, 2 and 7; section G); *d*, duration of the test shocks (fig. 6; section D); *e*, interelectrodal distance for the test stimuli (section F).

The changes of excitability are due to modifications of both the voltage and the time parameters of the voltage-capacity curves (fig. 6).

The distribution of effects along the nerves may be more complicated than has been assumed hitherto (fig. 7).

For a given application of d.c. the change at the anode does not permit predicting the effect at the cathode, and *vice versa* (fig. 5).   Similarly, the

after-effects at either pole cannot be predicted from the effects seen during the application (fig. 8).

The results are discussed in relation to the problem of the action of d.c. upon nerve and in relation to theories of electrical excitability.

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THE RESPIRATION OF ISOLATED LIVER AND KIDNEY TISSUES FROM ADRENALECTOMIZED RATS

S. R. TIPTON

From the Department of Physiology, The Ohio State University, Columbus

It is generally recognized that the energy necessary for the maintenance of the normal activities of liver and kidney tissues, and presumably of all tissues in the animal body, arises from oxidation-reduction processes chiefly. It is known now that, as a rule, electrons are transferred through a long chain of dehydrogenases through reversible redox systems from the oxidizable substance to molecular oxygen (1). In animal tissues the rate of oxidations is controlled not only by conditions which ordinarily affect the rate and equilibrium point of chemical reactions, but also by the influence of hormones. The specific nature of the latter factor is not very well understood. Damage of the enzyme systems concerned in oxidation must be an important factor in the development of many abnormal conditions of which adrenal insufficiency may be one.

Many abnormal conditions have been found to affect oxidations in the cells. Barron (2) has shown that Rous chicken sarcoma and also infectious myxoma in the rabbit do not oxidize succinic acid. It was further found that many tissues under the influence of filterable virus diseases oxidize it more slowly than normal tissues. Liver slices from rabbits suffering from diphtheria toxemia show a diminution in the power to oxidize lactic and pyruvic acids (3). The marked neural and muscular disturbances that are common to adrenal insufficiency in all species so far studied, together with the depression in basal heat production (the BMR may fall as much as 25 per cent below normal as crisis develops) which occurs at least in rats (4), suggest very strongly that oxidative systems may be adversely affected in the absence of the adrenal cortical hormones. A recent report by Crismon and Field (5) suggest that this may be particularly true of kidney tissue.

The results here reported show that adrenal insufficiency in rats results in a significant depression of the respiration of liver and kidney slices developing about a week after the operation, associated with a considerable diminution in the rate of oxidation of pyruvate and succinate by liver tissue.

METHODS. *Condition of the animals.* Young male rats weighing from

85 to 100 grams were selected for the tests. Litter mate controls were used where possible but the control group includes some animals who have no litter mates in the experimental group. The same is true of the experimental group. Bilateral adrenalectomy was carried out at a single operation. In our hands the average survival time of rats after adrenalectomy is 11 days. Consequently the measurements of oxygen consumption were made on the 7th to 10th day following the operation. The tested animals usually showed obvious signs of insufficiency.

The data on the experimental rats are taken only from animals which showed no accessory bodies at autopsy and a progressive loss in weight (as compared to controls) after the fourth day after operation. Sham operations were carried out on some controls. After recovery there was no appreciable difference from the other controls so this procedure was not generally followed.

In general the adrenalectomized animals received no hormonal or salt treatment. For some tests, however, two variations were made: a, some of the adrenalectomized rats were given Rubin-Krick's solution (6) as drinking water, and b, some of the control animals were fed the same quantity of food which their adrenalectomized litter mates consumed voluntarily.

Determination of Q_{O_2}. In general the same procedure was followed as described in a previous paper (7). The tissue slices were sectioned with a double blade cutter (8) adjusted to give a section thickness of less than 0.3 mm. Such a thin section is particularly necessary in determining accurately the high oxygen consumptions resulting from succinate oxidation. The kidney slices were sections taken from the central part of the kidney passing through cortex and medulla.

Sodium pyruvate, sodium succinate, and dextrose in concentrations of 0.04 M. previously buffered at pH 7.3 were added to the Ringer's solution at the beginning of those experiments designed to test the ability of the tissues to oxidize these metabolites. Oxygen consumption and CO_2 production (in tests where the R.Q. was measured) were determined in differential microvolumeters. The Q_{O_2} as usual is expressed in cubic millimeters per milligram dry weight per hour and is given as a positive figure. The suspension medium was Ringer's solution buffered at pH 7.3 with 0.02 M. phosphate buffer.

All tests were made with the animals under basal conditions.

RESULTS. *Liver.* Table 1 presents all the data obtained on the respiration of liver slices from normal and adrenalectomized rats. The "fed normals" were placed on the ordinary stock diet and allowed to eat all that they desired. The "partially-starved normals" were fed the same quantity of food that adrenalectomized litter mates ate voluntarily. Insufficiency is characterized by anorexia so the litter mate control was

starved to some extent. The "adrenalectomized" group received all the food that they desired and tap water for drinking. The "salt-treated adrenalectomized" received Rubin-Krick's salt solution (6) as drinking water from the time of operation until the respiration test. The tests were made from 7 to 10 days after the operation. The exact time depended upon the condition in which the animal appeared to be; that is, how close it was to collapse.

Adrenalectomy decreases the rate of oxygen consumption of liver slices from a mean value of 8.68 c.mm./mgm./hr. for the normals to 5.86

TABLE 1

The oxygen consumption of liver tissue from normal and adrenalectomized rats under basal conditions

The effect of different metabolites is also given

		CONTROL	SODIUM PYRU-VATE, 0.04 M	SODIUM SUC-CINATE, 0.04 M	DEXTROSE, 0.04 M
	TIME AFTER DEATH				
Fed normals (20 animals)					
		Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}
Mean	1st hour	8.68 ±0.15*	14.01 ±0.53	17.27 ±0.53	9.48 ±0.21
	2nd hour	8.11 ±0.12	13.7 ±0.47	16.93 ±0.42	9.02 ±0.19
	R.Q. =	0.61 ±0.01			
Partially-starved normals (3 animals)					
Mean	1st hour	7.95	13.0	15.45	8.65
Adrenalectomized (20 animals)					
Mean	1st hour	5.86 ±0.12	7.13 ±0.17	9.88 ±0.34	6.38 ±0.10
	2nd hour	5.47 ±0.11	6.88 ±0.14	9.45 ±0.29	6.16 ±0.12
	R.Q. =	0.64 ±0.02			
Salt-treated adrenalectomized (5 animals)					
Mean	1st hour	7.35	11.1	13.3	8.82

* Standard error of the mean.

for the adrenalectomized group. It will also be noted that the Q_{O_2} decreases in the second hour of the test to about the same extent in both normals and adrenalectomized groups. In the presence of substrate the decrease with time is not so marked. Partial starvation depresses the Q_{O_2} but to a smaller extent than does adrenalectomy. The liver slices from the salt-treated group have a rate of oxygen consumption approaching that of the partially-starved, unoperated animals.

As shown in table 1 pyruvate, succinate and dextrose (0.04 M) increase the oxygen consumption of liver tissue of well-fed and also of the partially-

starved normal rats. The Q_{O_2} of liver of the adrenalectomized group is stimulated also but as table 2 brings out, the percentage increase resulting from the addition of these substances is not so great as is the case in the unoperated normal group. Dextrose has the least effect, raising the Q_{O_2} in both normals and adrenalectomized rats about 10 per cent. Succinate shows the greatest effect producing a 100 per cent increase in normal rats but having a somewhat smaller influence on adrenalectomized animals. Very little of this decrease in rate of oxidation of succinate that is found in the operated rats can be attributed to starvation since, as table 1 indicates, the influence of succinate on the livers of partially-starved animals differs very little from that exerted on well-fed normals. Rosenthal (9) found that succinate oxidation was relatively independent of the nutritive condition of the rat. The effect of adrenalectomy suggests that in the absence of the cortical hormone the enzyme systems necessary for the

TABLE 2

The percentage change in oxygen consumption of the liver tissue resulting from the addition of metabolites as taken from data in table 1

TIME OF TEST	CONTROL	SODIUM PYRUVATE	SODIUM SUCCINATE	DEXTROSE
Normals				
	per cent	*per cent*	*per cent*	*per cent*
1st hour......................	0	+61.4	+98.9	+9.2
2nd hour......................	0	+68.9	+108.7	+11.2
Adrenalectomized				
1st hour......................	0	+21.6	+68.6	+8.87
2nd hour......................	0	+25.7	+72.7	+12.6

oxidation of succinic acid are depressed slightly. Von Szent-Györgyi (10) has shown that succinic acid and its oxidase are important components of the carbohydrate oxidation systems.

The rate of pyruvate oxidation by liver tissue is markedly decreased after adrenalectomy falling from 61.4 per cent stimulation in the normal group to 21.6 per cent in the animals having insufficiency of adrenal hormones. The oxidation of pyruvate in the latter group still proceeds actively but at a considerably slower rate than in normals. Pyruvate increases the respiration of liver of starved rats about as much as it does that of well-fed ones. In no case was there a depression of respiration as Rosenthal (9) found with pyruvate after long periods of starvation. Pyruvate is an important intermediary metabolite in carbohydrate and protein oxidation and a disturbance in its oxidation might result in an altered metabolism. However, the respiratory quotient does not differ

significantly in the normal and adrenalectomized groups (table 1). Apparently the residual pyruvate oxidation is adequate to maintain the same type of metabolism as is found in the normal rats. Salt-treated animals show values reverting toward the normal.

Kidney. Table 3 gives the results found for kidney tissue. Kidney tissue taken from rats suffering from adrenal insufficiency has a lower Q_{O_2} than the normal. The decrease shown here (18.66 c. mm./mgm./hr. to 13.73) agrees fairly well with the data recently reported by Crismon and Field for rats on the 7th and 8th days after operation.

TABLE 3

The oxygen consumption of kidney tissue from normal and adrenalectomized rats under basal conditions

NORMALS		ADRENALECTOMIZED	
Animal	Q_{O_2}	Animal	Q_{O_2}
STK1	18.5	STK1a	14.5
STK2	20.5	STK2a	12.7
STK2b	19.65	STK2c	15.3
STK3	17.55	STK3a	13.8
STK4	17.83	STK4a	10.73
STK4b	18.2	STK4c	13.5
STK5	19.3	STK5a	14.75
STK6	18.42	STK7	16.0
STK8	19.73	STK8a	13.22
STK9	17.34	STK9a	12.8
Mean............	18.66 ±0.31*		13.73 ±0.45

* Standard error of the mean.

Discussion. It has been shown that the oxygen consumption of liver and kidney slices is depressed in the later stages of adrenal insufficiency. Since summated tissue respiration amounts to at least 66 per cent of the basal oxygen consumption of the whole animal (11) the decrease in kidney and liver respiration might account for a significant part of the fall in basal metabolism which occurs in adrenalectomized rats. The fact that brain (7, 5) and skeletal muscle (5) show little change in oxygen consumption even in the late stages of adrenal insufficiency (the same has been found in this laboratory to be true of diaphragm muscle), suggests that a change in functional metabolism is also an important factor in the depression of the metabolic rate. It seems surprising that tissues such as brain and skeletal muscle which show such marked changes in functional activity after adrenalectomy should manifest such stable oxidative systems.

Substrate concentration may be a factor in the depression of Q_{O_2} of liver in adrenalectomized rats. In table 1 it will be noticed that the liver

Q_{O_2} is lower in partially-starved rats than in well-fed normals and it is restored by adding pyruvate or succinate to the medium.   The adrenalectomized rat suffers considerable anorexia and the resulting subnutritional state may act to lower the rate of oxygen consumption of the tissue.

Marked depression of the rate of oxygen consumption in adrenalectomized rats does not develop until about the sixth day after operation, or possibly the fifth (5).   This, coupled with the finding that cortical hormone has no stimulating effect on the tissue respiration when added to the medium (the effect is one of depression actually (7)) raises the possibility that the results reported in this paper may be indirect consequences of cortical hormone deficiency and not direct ones.   The action may be on the liver and kidney through the thyroid-pituitary complex.

The pyruvate oxidation system in animal tissues is composed of a dehydrogenase, diphosphothiamine, alloxazine and cytochrome-oxidase. The fact that after adrenalectomy in rats there is still an active pyruvate oxidation indicates that there is no severe disturbance of the enzyme chain, that is, that the chain is still intact, but some one or more of the various components may be weak in concentration or partially blocked.   It may be possible to substitute ferricyanide as the oxidizing agent in place of cytochrome-oxidase (12) and thus determine whether that component has been affected.   At present it is impossible even to suggest where the deficiency may be.

The rate of oxidation of succinic acid by liver is less depressed than pyruvate oxidation by adrenalectomy.   If, as Rosenthal (9) suggests, the rate of succinate oxidation may be used as a measure of the minimum acitivity of the cytochrome-oxidase system, the implication is that cytochrome-oxidase is depressed to some extent by adrenalectomy.   The greater depression of the pyruvate oxidation indicates that some one or more of the other components in addition to cytochrome-oxidase are depressed.

Lyman and Barron (13) have found that the oxidation of pyruvate, succinate, and the synthesis of carbohydrate from pyruvate by kidney tissue is appreciably decreased when the kidney is damaged by diethylene glycol.   Evidence is given that the drug acts particularly on activating proteins (dehydrogenases).   Since renal malfunction is so marked in the secondary changes resulting from adrenal insufficiency it seems desirable to determine the nature of the enzyme deficiency causing the respiratory depression shown in table 3.

SUMMARY

' Adrenalectomy results in a decrease in the Q_{O_2} of liver slices from a mean value of 8.68 found in well-fed normal rats to a mean value of 5.86 and a decrease in Q_{O_2} of kidney slices from a mean of 18.66 to 13.75.   The

decrease in respiration is shown 7 to 10 days after the operation. The type of substances metabolized is unaffected by adrenalectomy since the respiratory quotient remains unchanged. Liver slices show a decrease in the ability to oxidize pyruvate (a decrease of 40 per cent) and succinate (30 per cent) after adrenalectomy. The ability of the liver slices from both normal and adrenalectomized rats to oxidize added dextrose is slight. The respiratory depression is less when the operated animals are given salt in the drinking water.

The Q_{O_2} of liver slices from partially-starved, otherwise normal rats is decreased as compared with that of well-fed normal controls. The oxidation of pyruvic acid and of succinic acid shows little dependence on the state of nutrition of the animal.

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EFFECT OF ABLATING THE PYRIFORM-AMYGDALOID AREAS AND HIPPOCAMPI ON POSITIVE AND NEGATIVE OLFACTORY CONDITIONED REFLEXES AND ON CONDITIONED OLFACTORY DIFFERENTIATION[1]

WILLIAM F. ALLEN

From the Department of Anatomy, University of Oregon Medical School, Portland

The effects of extirpating the motor cortex, the prefrontal areas, the hippocampi and large portions of the occipito-parieto-temporal lobes exclusive of the pyriform areas and amygdaloid nuclei on acquiring and acquired olfactory conditioned reflexes has been reported previously. This, a continuation of the general olfactory problem, is concerned with the removals listed in the title on conditioned olfactory reflexes. All animals were subjected to the following tests: *a*, establishment of a conditioned foreleg response to clove vapor; *b*, ability to transfer this reflex to the opposite foreleg; *c*, ability to establish an absence of foreleg response to asafetida and differentiation between two olfactory conditioned reflexes, which means the dog must decide in 7 seconds whether to respond positively or negatively to these vapors; *d*, ability when blindfolded to go to a certain pan by smell and select and open a paper package containing meat from 3 paper packets of like size and texture.

Procedure. The apparatus used for recording the results, the mode of giving the inhalations, the anatomical and physiological controls used, together with a classification of the vapors as to whether they were affective over the olfactory nerve or over both the olfactory and trigeminal, have been fully recorded in earlier papers.

Operations. All extirpations were made from the temporal approach, usually one side at a time, at intervals of about two weeks.[2] Two methods were used for eliminating the pyriform-amygdaloid areas. 1. It was accomplished by use of a specially bent spatula or a loop of steel wire. 2. Utilization of a previous finding that the parieto-occipital cortex was not essential for any of the factors entering into this problem, a dorso-ventral slit was made through the gyrus suprasylvius to the depth of the

hippocampus. The handle of the scalpel was then passed ventrad along the outer surface of the hippocampus until it hit the base of the skull. Upon moving the tip forward, to the side and backward this area was either completely removed or there remained medially a narrow longitudinal strip of pyriform-amygdaloid area which was functionless. All that is essential for this problem is severance of all fiber connections from this area to isocortex (non-olfactory cortex) and to lower levels. Method 2 proved very satisfactory and was used for all dogs listed in this series except no. 4. If the hippocampus was also to be removed, the ventral half was extirpated with a small hooked knife and the dorsal half by a spatula.

PYRIFORM AMYGDALOID ABLATIONS. Complete removal of the pyriform lobes or areas without injury to the amygdaloid nuclei or their connecting fibers to the isocortex is very difficult if not impossible. By this area is meant an important olfactory complex (previous paper, fig. 2) consisting of the pyriform lobes and the cortical or most lateral of the amygdaloid nuclei.

Effect on dogs previously conditioned. It can be stated that before operating every dog of this group was able to respond correctly to cloves and asafetida 90 per cent of the time or better irrespective of whether the sequence was 5 cloves to 1 asafetida, 3 to 1, 1 to 1 or 1 to 5. In all dogs failure to respond to cloves was punished by shock. For asafetida the procedure differed—an absence of response in dogs 1 to 4 was rewarded by withholding the shock; while the positive responses in dogs 5 to 9 were punished by whipping, cuffing or scolding depending on the irritability of the dog.

A summary of the more important data obtained from this group of dogs after operating appears in table 1. After unilateral extirpations column 1 of the table discloses that all of the dogs but no. 4 differentiated conditionally between cloves and asafetida. All but dog 4 were also able to differentiate correctly between cloves and anise or xylol.

After bilateral extirpations, columns 2 and 3 of table 1 reveal no especial delay in the appearance or establishment of the positive response to cloves. In most instances it came with the first or second trial. Columns 4 to 8 show that bilateral ablation of the pyriform-amygdaloid area eliminates all olfactory conditioned differentiation with the first foreleg tested for at least 7 seconds when the clove and asafetida vapors were given in the same and even more varied orders of sequence than were used before operating. Ordinarily both clove and asafetida tests ran very strongly positive, the ratio of positive to negative responses being about the same for either vapor. This means for cloves that the ratio of responses to no responses is about the same as before operating; while for asafetida this ratio is practically reversed. Dog 8, however, revealed a

considerable number of failures to respond for both cloves and asafetida, but the ratio of positives to negatives is about the same for each.   Con-

TABLE 1

Effect of ablating the pyriform areas and amygdaloid nuclei on acquired olfactory reflexes

DOG	DIFFEREN-TIATION AFTER FIRST EXTIRPATION	First clove response came with test number	Clove reflex established with test number	AFTER BILATERAL EXTIRPATIONS									
				First foreleg					Second foreleg				
				Differentiation tests					Differentiation tests				
				Number of clove reflexes positive	Number of clove reflexes negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates	Number of clove responses positive	Number of clove responses negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates
1	Yes	1 L	1	287	15	81	7	No	141 R	10	37	2	No
2	Yes	1 L	1	295	13	60	4	No	23 R	2	5	0	No
3	Yes	1 R	4	72	7	13	1	No	50 L	4	6	8	Poorly
4	No	31 R	42	93	7	16	2	No	47 L	3	13	1	No
5	Yes	2 L	2	195	12	57	7	No					
After several series of asafetida tests				84	18	26	3	No					
6	Yes	2 R	2	189	11	61	4	No	43 L	8	3	19	Yes
After several series of asafetida tests				90	6	28	2	No					
After differentiation was established in left leg		7 R	16	100	0	18	0	No					
7	Yes	3 R	5	309	32	87	8	No					
8*	Yes	1 L	1	148	65	33	16	No					
9	Yes	1 L	1	217	35	70	18	No	50 R	0	10	0	No

R or L denotes order in which forelegs were tested.
* Dog died from exposure.

cerning the second foreleg tested, columns 9 to 13 show dog 6, still able to differentiate excellently with the left foreleg, the ratio of clove positives

to negatives being 43 to 8 and for asafetida 3 to 19. To determine if this dog was still unable to differentiate with the first foreleg tested, several series of differential tests were run off with this (right) foreleg, which resulted (table 1, bottom row for dog 6) in a total inability to hold back its foreleg responses for asafetida and discloses even less ability to differentiate than exhibited in the first tests (top row for dog 6). There is also the possibility that dog 3 was able to differentiate poorly with the second (left) foreleg, for columns 9 to 12 show the ratio of cloves to be 50 to 4 and for asafetida 6 to 8. It is of especial importance to record here that a later section on the brains of this group of dogs shows the contralateral ablations incomplete on these two dogs which differentiated with one foreleg.

All of the dogs which were unable to differentiate conditionally between cloves and asafetida after extirpation of the pyriform-amygdaloid areas were likewise unable to refrain from responding positively to other olfactory stimulating vapors such as anis and xylol when these were substituted for asafetida in differential tests with cloves. It should also be noted that bilateral removal of the pyriform-amygdaloid areas in no way interfered with the dog's ability to transfer the conditioned clove response from one foreleg to the other.

After being unable to obtain conditioned olfactory differentiation with the first foreleg tested following bilateral extirpation of the pyriform-amydgaloid areas, dogs 5 to 9 were subjected to the procedure instigated in a previous study of the prefrontal areas. This consisted of attempts to first reëstablish the negative conditioned reflex by giving only asafetida inhalations and punishing each positive response. If this reflex could be established then additional differential tests would be undertaken.

It will be seen from these tests, as in the preceding ones on the prefrontal areas, that the temperament of the dog played an important rôle on the ability of the dog to refrain from responding to asafetida. Dog 7 of the cocker spaniel variety typifies a group that was high spirited, quick of response and after the operations invariably responded positively to asafetida in the differential tests in spite of severe punishment. Out of 205 consecutive tests made on this dog for asafetida, only 4 were negative and no two came in succession. Although further differential tests were not warranted for this dog, a few were made, using cloves against asafetida or anis or xylol and resulting in only positive responses for each vapor. Dog 5, a shepherd, illustrates another type, which while equally alert was strongly inhibited by the light punishment inflicted for positive responses to asafetida. This type readily acquired a negative response to asafetida when this vapor was tested by itself. In dog 5 the first absence of response came with the 7th trial and from then on was fairly regular but not perfect until the 45th trial. In the tests for differentiation which followed, the first trial for cloves and two others out of the first five were

positive, the first asafetida and the following clove tests were both negative. A summary of these tests (table 1, lower column for dog 5) shows 84 positives and 18 negatives for cloves and for asafetida 26 positives to 3 negatives, which demonstrates a greater ratio of failures to respond for cloves than for asafetida. Dog 6, a greyhound, had more difficulty than dog 5 in regaining the absence of response to asafetida when tested in series, but in the differential tests which followed (table 1, lower column for dog 6) there were proportionately about the same number of positive responses for asafetida as for cloves.

An attempt was made in several of these dogs to differentiate between cloves positively and acetic acid (chiefly an irritant) negatively with little success, which suggests that the general cutaneous fibers to the cortex were severed in the operations.

After learning that a paper package might contain meat these dogs were able when blindfolded to go to a pan and instantly select a paper package containing meat from 3 paper packages of like size and consistency. Their responses to solutions of sugar, salt, quinine and acetic acid dropped on their tongues were the same as normal dogs.

A summary of the autopsy records and studies made on formalin prepared brains of this group of dogs often revealed the presence of a narrow median longitudinal strip of pyriform-amygdaloid cortex, which ranged in width from 2 to 3 mm. in some instances to 4 and 5 mm. in others. In dog 9 the remnant on the right side was 5 to 6 cm. wide. These remnants were usually yellow in color, sometimes of soft texture and all fiber connections to the isocortex were severed. Histological sections made of several of them indicated incapability of functioning. A complete removal of the pyriform-amygdaloid area was accomplished on the left side of dogs 2, 3, 6 and 9 and on the right side of dogs 4 and 8.

Dogs 3 and 6 which had the pyriform-amygdaloid areas completely extirpated from the right side possessed median longitudinal remnants of considerable width on the left side. In dog 3, the pyriform cortex was intact with the isocortex caudally, but severed elsewhere. In dog 6, the longitudinal remnant was 8 to 9 mm. wide and connected with the isocortex cephalad and caudad. Histological sections of both remnants revealed many normal cells in the regions which were connected to the isocortex. The presence of these large remnants of the right pyriform areas and their probable connections with the isocortex in dogs 3 and 6 can readily explain how these two dogs were able to respond correctly at times with their left legs to cloves and asafetida. The anterior commissure of dog 4 was sectioned after the Marchi method and found to be filled with degenerated fibers, but the same may be said of other dogs which were able to differentiate correctly after unilateral extirpations of the opposite pyriform-amygdaloid area.

With the exception of dog 1, paralyzed in the right leg, there was only

slightly more or no more than normal degeneration in Marchi sections of the pyramids. There was always more than the normal amount of degenerated fibers in the fornices of Marchi sections through the hypothalamus.

Effect on conditioning. The procedure was identical to that used in the previous section except that removal of the pyriform-amygdaloid areas preceded all attempts at conditioning. Table 2 summarizes the effects of the lesions on three of the tests that were used on the animals previously conditioned. Columns 1 and 2 of this table show that these dogs experienced little, if any, difficulty in acquiring the positive conditioned response for cloves. The apparent delay in dog 12 for the first foreleg tested can readily be explained by the fact that this leg was paralyzed, but when established the reflex became very regular. Columns 8 and 9

TABLE 2

Effect of ablating the pyriform areas and amygdaloid nuclei on acquiring olfactory conditioned reflexes

DOG	FIRST FORELEG TESTED (RIGHT)							SECOND FORELEG TESTED (LEFT)						
	First clove response came with test number	Clove reflex established with test number	Differentiation tests					First clove response came with test number	Clove reflex established with test number	Differentiation tests				
			Number of clove responses positive	Number of clove responses negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates			Number of clove responses positive	Number of clove responses negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates
10	36	75	288	13	78	6	No	20	98	150	0	30	0	No
11	12	31	266	34	54	6	No	9	37	24	1	5	0	No
12	48	257	116	9	20	0	No	31	32	100	0	20	0	No

Dog 12 paralyzed in right foreleg.

indicate a few more than the normal number of tests were required to transfer the clove reflex from one foreleg to the other. It is apparent from columns 3 to 7 and 10 to 14 that these dogs were unable to acquire an absence of response with either foreleg for asafetida when mixed with clove inhalations in ratios of 5 cloves to 1 asafetida, 3 to 1, 1 to 1 or 1 to 5. It made no difference whether the ablations were made in one operation, dog 10, or in two operations, dogs 11 and 12.

The autopsy records and studies made from formalin prepared brains revealed the presence of median longitudinal strips of pyriform-amygdaloid cortex ranging in width from 1 to 2 mm. to 3 to 5 mm. These remnants had a yellow color and all connections with the isocortex were severed. Histological sections of the remnants of dogs 11 and 12 demonstrated that the cells of the lobe and nucleus were incapable of functioning.

Marchi sections through the medulla showed complete degeneration of the fibers of the left pyramid of dog 12 but insufficient in the other pyramids to block impulses.   Marchi sections through the hypothalamus revealed slightly more than the normal number of degenerated fibers in the fornices.

All 3 dogs were able to select a paper package containing meat from 3 paper packages of like size and texture.   Their taste responses were normal to solutions of sugar, salt, quinine and acetic acid dropped on their tongues.

PYRIFORM-AMYGDALOID AND HIPPOCAMPAL EXTIRPATIONS.   Although an earlier report demonstrated that the hippocampi could be removed without

TABLE 3

Effect of ablating the pyriform areas, amygdaloid nuclei and hippocampi on acquired olfactory reflexes

DOG	DIFFEREN-TIATED BEFORE OPERA-TIONS	AFTER LEFT SIDE EXTIRPATIONS			AFTER RIGHT SIDE EXTIRPATIONS						
							Differentiation tests				
		First clove response came with test number	Clove reflex established with test number	Differentiates	First clove response came with test number	Clove reflex established with test number	Number of clove reflexes positive	Number of clove reflexes negative	Number of asafetida reflexes positive	Number of asafetida reflexes negative	Differentiates
13	Yes R	25 R	51	Yes	1 R	1	194	8	36	4	No
14	Yes R	1 R	1	Yes	1 R	1	166	27	42	10	No
15	Yes R	3 L	3	Yes	1 L	1	71	9	28	3	No

R or L signifies the foreleg tested.
Right foreleg of 13 was paralyzed.

affecting the positive clove response or the correct responses for cloves and asafetida when mixed in various ratios and the previous section showed that eliminating the pyriform-amygdaloid areas did not interfere with the clove reflex it seemed possible that deleting both of these important anatomical areas for olfaction might block or seriously interfere with the positive conditioned response for cloves.   This was accomplished in 3 dogs, using the second method for eliminating the pyriform-amygdaloid areas.

Effect on acquired reflexes.   Column 1 of table 3 indicates that before operating dogs 13 to 15 could respond correctly 90 per cent of the time to cloves and asafetida in 7 seconds when the ratio of inhalations was 5 cloves to 1 asafetida, 3 to 1, 1 to 1 and 1 to 5.   All clove errors were punished by

shock and all asafetida by a whip or cuff.   Columns 2 and 3 reveal that
the same ability to respond correctly to cloves and asafetida continued
after a contralateral ablation for dogs 13 and 14 and a homolateral ablation
for dog 15.   The delayed appearance of the reflex in dog 13 was caused by
paralysis.

  After bilateral ablations it is obvious from columns 5 and 6 of table 3
that the second lesion, made on the right side, in no way affected the clove
response.   It came with and became constant with the first trial.   As
was to be expected from the previous experiments their ability to dif-
ferentiate correctly between cloves and asafetida in 7 seconds was com-
pletely lost.   Since dog 15 never made any unnecessary movements and
his responses to both cloves and asafetida were more deliberate than dogs
13 and 14 he was given an additional series of differential tests after having
quickly obtained an absence of response to asafetida when this vapor was
tested continuously.   The second differential tests were identical to the
first in showing no signs of conditioned differentiation.

  After learning that a paper package might contain meat all of these
dogs were able when blindfolded to find a pan and select from it a paper
package containing meat from 3 paper packets of like size and texture.
Their taste responses to solutions of sugar, salt, quinine and acetic acid
dropped on their tongues were unchanged after the lesions.

  Autopsy and examination of formalin prepared brains of this group
demonstrated complete removal of the pyriform-amygdaloid area and
hippocampus from the right side of dog 15.   There remained on both
sides of dogs 13 and 14 median longitudinal strips of the pyriform-amygda-
loid cortex 2 to 5 mm. wide.   There was also a similar remnant on the left
side of dog 15.   All of these remnants were yellow in color or soft in texture
and all connections with the adjacent isocortex were obviously lost.
There can be no question but that the hippocampi were also functionally
eliminated.   In dogs 13 and 14 and on the left side of dog 15 about 5 mm. of
the extreme ventral tips of these structures remained at autopsy, which
means that about 90 per cent including the fornix was removed, and of the
remnant at least one-half was yellow in color.

  Marchi sections of the medulla revealed very little more than the normal
number of degenerated fibers in the pyramids, except in the left of dog 13,
which would account for the paralysis of the right leg.

  DISCUSSION.   The complicated anatomical relationships of the central
olfactory system as worked out by Cajal, van Gehuchten, Johnston and
others and shown in figure 2 of the previous paper can be summarized
as follows: 1. Olfactory impulses entering the olfactory bulbs are dis-
tributed to 3 primary areas.   2. These areas supply a primitive median
component, the hypothalamus and a more lateral component, the pyriform-
amygdaloid complex or area.   3. The hypothalamus furnishes the chief

olfactory reflex connections to lower levels and the pyriform-amygdaloid area the chief afferent olfactory connections to the isocortex (general or non-olfactory cortex).   Attention, however, is called to one significant exception originating from the hypothalamus, namely, the mammillo-thalamic bundle which sends many fibers to the anterior thalamic nucleus where olfactory impulses may be relayed to the cerebral cortex.

Concerning the evolution of the olfactory system Johnston has clearly shown that the pyriform lobes and the most lateral of the amygdaloid nuclei (cortical nucleus) have developed late phylogenetically as a probable result of increased needs from a changed environment from water to land. Johnston calls attention to the interconnections between the lateral portion of the amygdaloid nucleus and the pyriform lobe and from these areas the isocortex of the same and opposite sides; the latter were said to be short and long association fibers, external capsule fibers and anterior commissure fibers.   He ventured the opinion that the pyriform lobe and the lateral or cortical amygdaloid nucleus are associated with olfactory-somatic correlation and the more median amygdaloid nuclei are associated with olfactory-gustatory correlation.

The earlier literature on the effects of decortication and temporal lobectomy on olfaction have been considered in earlier papers.   More recently Dusser de Barenne, and Bard and Rioch have reported olfactory behavior after removals of the cerebral cortex; while Klüver and Bucy have noted strong oral tendencies (licking, chewing, touching of the lips, "smelling") after temporal lobectomy.

The writer agrees with Papez that no (recent) evidence has been pre-sented to demonstrate that the hippocampi are important structures for olfaction.   However, none of the writer's dogs with deleted hippocampi showed any signs of having lost a "central emotive center" as postulated by Papez in 1937.

Unfortunately the writer has been unable to see some of the early Russian work on olfactory conditioned reflexes, but according to Zavad-sky's review it cannot be taken seriously.   Zavadsky's contribution con-sisted of removing the pyriform lobes and some of the adjacent hippocampus and isocortex from 2 dogs.   He afterward obtained a con-ditioned salivary reflex from inhaling camphor and reflex movements of the nostrils and secretions of saliva from the smell of meat powder.   Zavad-sky like several others was unfortunate in the selection of his olfactory stimulating vapor, for the writer demonstrated (1937) that a conditioned reflex could be obtained from camphor after the olfactory nerves had been cut and is consequently a trigeminal as well as an olfactory stimulant.

It is obvious from this investigation that a positive conditioned foreleg response to clove vapor is readily acquired and not lost from bilateral elimination of the pyriform areas, amygdaloid nuclei and 95 to 100 per cent

of the hippocampi and fornices. The real effects from these bilateral extirpations came with the conditioned olfactory differential tests, when it was shown that removal of the pyriform-amygdaloid areas with or without the hippocampi prevented correct conditioned differentiation in 7 seconds between cloves and asafetida or cloves and anis or xylol when clove vapor was given to the others in ratios of 5 tests to 1, 3 to 1, 1 to 1, and 1 to 5. These results were the same whether the tests followed or preceded and followed the operations, or whether the removals were made in one or two operations.

As reported for deletion of the prefrontal areas the temperamental differences of the dogs played a considerable rôle in the character of the responses after extirpation of the pyriform-amygdaloid areas. This was especially noticeable in the serial tests for obtaining absence of response to asafetida. In spite of severe punishment the high spirited dogs continued to respond positively to asafetida, while the more quiet dogs readily acquired an absence of response, but when asafetida tests were afterward mixed with clove, there was no sign of conditioned olfactory differentiation between these vapors with either group.

Two observations have come out of this study in non-support of the pyriform-amygdaloid-hippocampal areas being the centers or at least the sole centers for the detection of minute differences in odors. They are: 1, after the operations these dogs were able by smell to select a paper package containing meat from 3 paper packets of like size and texture; 2, following an erroneous response to cloves or asafetida they may brace themselves or cry as if in expectancy of punishment that is to follow.

Brown and Ghiselli have recently reported olfactory discrimination to be subcortical in rats, but as was previously pointed out (1938) for Swann's work, they used an olfactory stimulating vapor against creosote, an irritant, effective over both the trigeminal and olfactory nerves.

It was first supposed after finding the Marchi sections of the anterior commissure of dog 4 (only dog not able to differentiate conditionally after unilateral deletion of the pyriform-amygdaloid area) full of degenerated fibers that severance of this bundle might have blocked all of the crossed afferent impulses in this dog and thus prevented conditioned olfactory differentiation in the contralateral leg. However it was found later that Marchi sections of the anterior commissure from other unilateral lesion dogs, which differentiated conditionally, were also filled with degenerated fibers.

Since deletion of the pyriform-amygdaloid areas produced the same interference with conditioned olfactory differentiation as resulted from removal of the prefrontal areas, their anatomical relationships would suggest that the pyriform-amygdaloid extirpations cut off the normal afferent supply of olfactory impulses to the cortical areas concerned with

conditioned olfactory differentiation in 7 seconds, a very high order of olfactory synthesis.

Likewise from the same order of reasoning the complete series of studies suggests that the afferent olfactory impulses which go to the isocortex concerned with the production of a conditioned olfactory response ordinarily travel by way of the mammillo-thalamic bundle, anterior thalamic nucleus and internal capsule.

All that can be claimed for these and the previous olfactory studies is that they represent the normal mode of operation during the interval of experimentation after operating, which varied from 6 weeks to 4 months. It is possible that the isocortex may possess inactive areas or have other olfactory connections which may at some future time take over the functions of the destroyed areas.

SUMMARY

To determine the effects of certain temporal lobe lesions on olfaction, the following tests were made after and before and after the operations: a, establishment of a conditioned foreleg reflex to clove vapor; b, transference of the clove reflex from one foreleg to the other; c establishment of a negative conditioned reflex (absence of foreleg response) for asafetida and conditioned differentiation, which involved a decision in 7 seconds whether to respond positively or negatively to the vapor inhaled, irrespective of any order in which it might come; d, food from no food discrimination by smell.

With one exception, considered in the discussion, unilateral extirpation of the pyriform-amygdaloid areas produced little or no effect on a, b, c or d with either the contralateral or the homolateral foreleg.

Bilateral extirpation of the pyriform-amygdaloid areas or severance of their isocortex connections had little or no effect on a, b or d, but abolished c in every instance in which elimination was complete.

The inclusion of the hippocampi (95 to 100 per cent) to the pyriform-amygdaloid ablations resulted in no additional effects on a, b or d.

Other purely olfactory vapors such as anis and xylol were substituted for asafetida in the differential tests with the same results.

Consideration was given in the discussion to: 1, the relationship of the pyriform-amygdaloid areas to the afferent side of conditioned olfactory differentiation; 2, certain temperamental variations noted in c; 3, the likelihood of the mammillo-thalamic bundle furnishing important afferent olfactory impulses for a conditioned reflex.

Deletion of the pyriform-amygdaloid-hippocampal areas produced no effect on the taste responses elicited from solutions of sugar, salt, quinine and acetic acid dropped on the tongue.

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CIRCULATION TIME THROUGH AERATED AND ATELECTATIC LUNGS IN DOGS AS DETERMINED BY THE USE OF SODIUM CYANIDE

JOHN L. KEELEY

From the Laboratory of Surgical Research, Harvard Medical School, Boston, Mass.

Stewart's formula (1) V equals $Q\ \dfrac{60}{T}$, which concerns the pulmonary circulation, may be applied to the circulation through one lung. Thus V' equals $Q'\ \dfrac{60}{T'}$ where V' is the minute volume flow of blood through one lung; Q' is the quantity of blood in one lung; and T' is the mean blood velocity through that lung in seconds. V' and Q' are decreased during atelectasis (2, 3, 4) but no report was found in which both V' and Q' had been determined in the same atelectatic lung thus permitting calculation of T'. Similarly, no direct study of the effect of atelectasis on the mean blood velocity through a lung was encountered in the literature. An index of the mean velocity of blood flow through the lungs may be obtained by measuring pulmonary circulation time (1, 5). In this study the circulation time through aerated and atelectatic lungs and through certain associated vascular circuits was determined by the use of sodium cyanide (6, 7).

MATERIAL AND PROCEDURE. Full grown healthy dogs were used. Under pentobarbital (Nembutal) anesthesia the chest was opened through the left fourth intercostal space. Throughout the experiments the intermittent intratracheal insufflation was adjusted to a minimum compatible with the abolition of spontaneous respiratory movements. The pericardium was opened and a loop of catgut was passed around the left branch of the pulmonary artery so that the right and left branches of the pulmonary artery could be supported while injections were being made into them. The cardiac rate and the rate of the respirator were determined with a stopclock.

Two series of observations were made, the first while both lungs were being aerated, and the second after atelectasis of the left lung had been caused by division and closure of the bronchus. In each series, adequate doses of sodium cyanide (0.18–0.25 c.c. of 0.5 per cent solution) were in-

jected into 1, the right branch of the pulmonary artery (R.B.P.A.); 2, the left branch of the pulmonary artery (L.B.P.A.); 3, the right ventricle (R.V.), and 4, the left ventricle (L.V.).   The interval between the injection of sodium cyanide and the occurrence of the end point was determined by a stopclock, and it represents the circulation time from the point of injection to the carotid sinus (8, 9).   From these results the circulation time through the lungs[1] was calculated.

RESULTS.   When both lungs were aerated by intermittent insufflation (table 1), the results were as follows: the L.B.P.A. to carotid sinus (C.S.) .

TABLE 1

Circulation time from site of injection to carotid sinus and the calculated pulmonary circulation time through the right and left lungs when both are aerated

| | TRIAL | CARDIAC RATE PER MINUTE | RESPIRATOR RATE PER MINUTE | CIRCULATION TIME FROM SITE OF INJECTION TO CAROTID SINUS IN SECONDS | | | | CIRCULATION TIME THROUGH RIGHT LUNG | CIRCULATION TIME THROUGH LEFT LUNG |
				L.B.-P.A.	R.B.-P.A.	R.V.	L.V.		
Experiment 1, 4/20/37	1	140	15	9.0	9.5	10.0			
Experiment 2, 8/11/37	1	·114	26	9.5	9.5	11.5	5.0	4.5	4.5
	2	114	26	8.5	8.0	12.0	5.0	3.0	3.5
	3	110	26	9.5	8.5	10.0	4.5	4.0	5.0
Experiment 3, 8/25/37	1	126	30	10.0	9.0	10.0	5.0	4.0	5.0
	2	120	30	10.0	10.0	10.0	5.0	5.0	5.0
	3	120	30	10.0	10.0	10.0	5.0	5.0	5.0
Experiment 4, 4/15/37	1	124	36	10.0	8.5				
Average..........		121	27	9.5	9.1	10.5	4.9	4.2	4.6

L.B.P.A. = Left branch pulmonary artery.   R.B.P.A. = Right branch pulmonary artery.   R.V. = Right ventricle.   L.V. = Left ventricle.

circulation time varied from 8.5 to 10 seconds with an average of 9.5 seconds.   The R.B.P.A. to C.S. circulation time was only slightly less, ranging from 8 to 10 seconds and averaging 9.1 seconds.   The circulation time for the vascular segment from the R.V. to the C.S. varied from 10 to 12 seconds with an average of 10.5 seconds.   The L.V. to C.S. circulation time averaged 4.9 seconds.   The pulmonary circulation time through the right and left lungs was obtained by subtracting the L.V. to C.S. circula-

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[1] The term, "circulation time through the lungs," used here is defined as the time calculated to be necessary for the passage of blood from either branch of the pulmonary artery to the left ventricle.

TABLE 2

Circulation time from the sites of injection to the carotid sinus and the calculated pulmonary circulation time through the right and left lungs when the right lung is aerated and the left lung is atelectatic

		CARDIAC RATE PER MINUTE	RESPIRATOR RATE PER MINUTE	CIRCULATION TIME FROM SITE OF INJECTION TO CAROTID SINUS IN SECONDS				NET CIRCULATION TIME THROUGH FUNCTIONING LUNG	NET CIRCULATION TIME THROUGH NONFUNCTIONING LUNG	REMARKS
TRIAL	TIME			L.B.P.A.	R.B.P.A.	R.V.	L.V.			
4/20/37: Experiment 1. Sex, male; weight, 22.7 kgm.; anesthetized with 25 cc. pentobarbital solution, 1:45 p.m.										
1	3:52	120	16	12.0	8.5	9.5	4.0	4.5	8.0	Lung collapsed and mottled
2	4:03	128	20	12.0	7.0	7.0	5.0	2.0	7.0	
3	4:13	112	24	10.5	6.5	7.5	5.0	1.2	5.5	
4	4:30	100	24	10.0	7.0	9.0	5.0	2.0	5.0	Lung purple
Average for group		115	21	11.1	7.25	8.25	4.75	2.4	6.3	
8/11/37: Experiment 2. Sex, female; weight, 17.4 kgm.; anesthetized with 18 cc. pentobarbital solution at 12:45 p.m.										
1	2:32	120	32	14.0	10.0	10.0	5.0	5.0	9.0	Lung collapsed; central portion "liver-like"
2	2:45	138	24	12.5	9.0	10.5	5.0	4.0	7.5	
3	2:55	128	30	11.0	8.0	10.5	5.0	3.0	6.0	
4	3:33	126	30	14.5	9.5	8.5	5.0	4.5	9.5	Lung entirely "liver-like"
5	3:40	120	30	14.0	9.0	10.0	5.5	3.5	8.5	Lung entirely "liver-like"
Average for group		126	29.2	13.2	9.1	9.9	5.1	4.0	8.1	
8/25/37: Experiment 3. Sex, male; weight, 30.3 kgm.; anesthetized with 31 cc. pentobarbital solution at 12:45 p.m.										
1	2:48	120	36	10.0	8.0	8.5	5.0	3.0	5.0	Lung collapsed; slight crepitus in peripheral portions
2	3:35	136	30	9.0	7.5	8.0	4.5	3.0	4.5	
3	3:45	134	36	11.0	8.0	8.0	5.0	3.0	6.0	Lung "liver-like"
4	4:05	114	36	10.5	8.0	9.0	5.0	3.0	5.5	Lung "liver-like"
5	4:15	132	36	11.5	8.0	10.0	5.5	2.5	6.0	Lung "liver-like"
6	4:40	114	36	10.5	8.0	10.0	5.5	2.5	5.0	Lung "liver-like"
Average for group		125	35	10.4	7.9	8.9	5.09	2.83	5.3	
4/15/37: Experiment 4. Sex, male; weight, 24.3 kgm.; anesthetized with 25 cc. pentobarbital solution at 2:00 p.m.										
1	3:10	132	48	11.5	6.5	7.0	5.0	1.5	6.5	Lung collapsed; central portion heavily mottled
2	3:25	136	40	10.0	6.5	7.0	4.5	2.0	5.5	
3	3:55	138	42	10.0	6.5	7.0	5.5	1.0	4.5	
4	4:22	138	42	10.0	7.0		5.0	2.0	5.0	Lung "liver-like" throughout
Average for group		136	43	10.4	6.6	7.0	5.0	1.6	5.3	
Total average		125.5	32.2	11.2	7.8	8.7	5.0	2.8	6.2	

L.B.P.A. = left branch pulmonary artery. R.B.P.A. = Right branch pulmonary artery. R.V. = Right ventricle. L.V. = Left ventricle.

tion time from the results obtained by injections into the branches of the pulmonary artery. The average circulation time through the right lung was 4.2 seconds, and the average circulation time through the left lung was 4.6 seconds, or 0.4 second longer.

Table 2 shows the results obtained after atelectasis of the left lung had been produced by division of its bronchus, closure of the divided ends, and subsequent absorption of the contained gases. The L.B.P.A. to C.S. circulation time ranged from 9 to 14.5 seconds, with an average of all these determinations through this circuit (part of which was composed of the atelectatic lung) of 11.2 seconds. The R.B.P.A. to C.S. circulation time varied from 6.5 to 10 seconds, with an average of 7.8 seconds. The R.V. to C.S. circulation time ranged from 7 to 10 seconds with an average of 8.7 seconds. The L.V. to C.S. circulation time in this series varied from 4 to 5.5 seconds and averaged 5 seconds.

By subtracting the L.V. to C.S. circulation time from the results obtained by injections into the branches of the pulmonary artery the pulmonary circulation time through the right and left lungs was obtained. The figures for the circulation time through the aerated lung varied from 1 to 5 seconds, and averaged 2.6 seconds. For the atelectatic lung the extremes of circulation time were 4.5 and 9 seconds, with an average of 6.2 seconds, or 3.4 seconds longer than the circulation time through the aerated lung.

DISCUSSION. Only two reports dealing with the pulmonary circulation time in dogs were found in the literature. Stewart (1) found the pulmonary circulation time in intact anesthetized dogs to average 8 seconds. He obtained this result by subtracting the left ventricle to femoral artery circulation time from the right ventricle to femoral artery circulation time. Kuno (10) using a canine heart-lung preparation found that the mean pulmonary circulation time averaged 2.8 seconds in five experiments. He called this interval the pulmonary artery to pulmonary vein circulation time. Because of the differences in the vascular segments studied and in experimental conditions and methods, no direct comparison can be made between the results of Stewart or Kuno and those presented in this report.

The possibilities of 1, inaccuracies in timing; 2, variations in the degree of atelectasis of the left lung, and 3, irregularities in the rate and extent of aeration of the right lung must be considered in these experiments. The end points caused by adequate doses of sodium cyanide are clear-cut even under anesthesia and continue to be so after repeated determinations. Errors in timing from this source therefore need not be considered. The relation of the time of injection to the phases of the cardiac cycle constitutes a source of error which becomes less important with increases in cardiac rate. With a cardiac rate which ranged between 100 and 138 per minute, the maximal delay in the right and left ventricles could not prolong the R.V. to C.S. circulation time more than approximately a

second.   The remaining three determinations were subject to a maximal prolongation of approximately a half-second, due to delay of the sodium cyanide in the left ventricle.   Calculation of the circulation time through the lungs by subtracting the L.V. to C.S. circulation time from the circulation time determined by injection into the branches of the pulmonary artery may be subject to no error if the delay in the left ventricle prolongs the two sets of determinations to the same extent.   The maximal error (approximately one-half second) in the calculated pulmonary circulation time would occur when the greatest possible delay in the left ventricle affected one determination and when there was no delay in the left ventricle to affect the other determination.   Stewart (1) has pointed out that when a mean of a number of successive determinations is taken, the error due to delay in the ventricle or ventricles is automatically approximated to the average.   Errors in timing due to the human element, if present, were small, as is indicated by the uniformity of the results in the L.V. to C.S. circulation time determinations, in which the effects of other variables were minimal.

A comparable degree of atelectasis of the left lung in all four animals was assured by the length of the intervals (thirty to forty-five minutes) between closure of the bronchus and the first determinations of the circulation time shown in table 2.   The fact that 79 per cent of the values for the L.B.P.A. to C.S. circulation time fell within plus or minus ten per cent of the average value of 11.2 seconds is further assurance that no great variation in the degree of atelectasis was present.

The wider range of figures for the R.B.P.A. to C.S. circulation time is probably due to several variable conditions such as the respirator rate, the degree of expansion of the right lung, and the length of time the increased intratracheal pressure was maintained, this latter situation being dependent on the proportion between the inside diameter of the trachea and the outside diameter of the intratracheal tube.

From the results of the experiments described here, it is seen that in a lung recently rendered atelectatic by the occlusion of its bronchus, the values for V' (the minute flow of blood through one lung) and Q' (the quantity of blood in one lung) are such as to consistently cause a decrease in T' (the mean blood velocity through the lung) as indicated by the pulmonary circulation time.   Conversely, when one lung has been rendered atelectatic, the minute volume flow of blood and the quantity of blood in the other lung are such that the circulation time through the aerated lung is consistently decreased.

<div align="center">SUMMARY</div>

1. By the injection of adequate doses of sodium cyanide into 1, the left branch of the pulmonary artery; 2, the right branch of the pulmonary artery; 3, the right ventricle, and 4, the left ventricle, the circulation time

from these sites of injection to the carotid sinus was determined in dogs with open chests, before and after division and closure of the left bronchus. From these results, the circulation time through the right and left lungs was calculated.

2. When both lungs were aerated, there was no great difference in the circulation time through the right and left lungs. The average calculated circulation time for the right lung was 4.2 seconds, and for the left lung it was 4.6 seconds.

3. When one lung was rendered atelectatic by the closure of its bronchus, the circulation time through the atelectatic lung was increased to an average value of 6.2 seconds, whereas the circulation time through the aerated lung was decreased to an average of 2.8 seconds.

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STIMULATION OF NERVES BY DIRECT CURRENTS

A. ROSENBLUETH

From the Department of Physiology in the Harvard Medical School

It is generally accepted that the nerve impulses elicited by the make of a direct current originate exclusively at the region of the nerve in contact with the cathode, while the impulses set up by the break of the current start exclusively at the anode. The statement implies two laws, one positive and the other negative, as follows: that the make starts impulses at the cathode and the break at the anode; and that the anode cannot stimulate when the current is closed, and the cathode when the current is broken.

A critical survey of the experimental basis for these laws (see Gotch, 1900, and Laugier, 1921, for summaries of this evidence) shows that, whereas the positive law is adequately supported by the data, the negative statement is based on the inference that stimulation by the make at the anode or by the break at the cathode is an unnecessary assumption—i.e., that the observations adduced in support of that assumption can be reasonably explained by the first law.

In a study of the changes of electrical excitability of nerve produced by applications of direct current (Rosenblueth, 1941) certain observations were made which did not fit reasonably with the classical concepts of nerve stimulation mentioned above. The present report deals with experiments made to test the validity of the negative statement in the classical law of electrical stimulation of nerve.

METHOD. The nerves studied were the cat's popliteal, peroneal and phrenic. The animals were usually anesthetized with dial (Ciba, 0.75 cc. intraperitoneally). Control experiments made on animals which had been decapitated 30 to 90 minutes previously, under ether, showed that the use of dial anesthesia led to only minor quantitative differences in the results.

The nerves were either *in situ*, with the circulation relatively intact, or else excised and placed in a moist chamber. The indicator of activity of the circulated popliteal nerves was the contraction of the muscles attached to the Achilles tendon. The legs were held firmly by drills inserted into the tibiae. The tendons were attached to a tension myograph recording on a kymograph. Upward excursions in the tracings denote contraction.

In the excised nerves the nerve impulses were recorded electrically from a cathode-ray oscillograph after suitable amplification. The details of the

different modes of recording adopted for different purposes will be described with the corresponding results.

When the popliteal nerves were *in situ* they were severed centrally, at the emergence of the sciatic from the pelvis. The electrodes were chlorided silver wires supported and shielded by split rubber tubing. The popliteal was usually separated from the peroneal at three places in the thigh, for spaces of about 1.5 to 2 cm. The rubber electrode holders, supporting 2 to 4 electrodes, were inserted through these spaces. Cotton was then packed lightly around the electrodes, providing relative insulation from the surrounding muscles. These muscles and the skin were then sewed up, leaving only the insulated leads of the electrodes to emerge. The nerves were therefore at normal temperature and protected from desiccation. Throughout the dissection damage of the blood vessels was carefully avoided.

The electrodes used for applying direct currents to the excised nerves were calomel half-cells. The bridge to the nerve was provided first by agar-Ringer and finally by the wicks, soaked with Ringer, upon which the nerves lay. Tests showed that within the range of voltages employed the electrodes were impolarizable. For recording the nerve responses no special precautions were usually taken with regard to the polarizability of the electrodes.

The two methods employed offered different advantages and disadvantages, respectively. The nerves *in situ* were in quite normal physiological condition. On the other hand, the non-polarizability of chlorided silver wires is only relative. Furthermore, the popliteal nerves as prepared had several points of intimate contact with surrounding tissues, mainly with the peroneal nerve. It is possible, therefore, that in certain observations the currents applied may have entered or left the nerves tested at other points than those in contact with the electrodes used. In the excised nerves the application of the currents could be rigorously controlled, but the physiological condition was unavoidably deeply disturbed.

The sources of d.c. were usually dry cells, the intensity being regulated by means of a potentiometer. It was found that ordinary spring keys are quite satisfactory for making the current—i.e., the difference of potential across the electrodes can be suddenly established and maintained. The release of such a key, on the other hand, was found quite unreliable for breaking the current suddenly, as evidenced by the irregularity of the responses to the opening of the currents. The procedure adopted was, therefore, to make the current by means of a key in the battery circuit ahead of the potentiometer regulating the voltage, and to break it by closing with a similar key a short circuit of negligible resistance placed between the potentiometer and the series resistances, electrodes and nerve.

The voltmeter with which the potential drops were measured was some-

times placed ahead of resistances added in series to the electrodes, or else it read the voltage drop across the electrodes or the nerve. An ammeter was also used sometimes to register the intensity of the currents flowing through the nerve.

In the excised nerves the d.c. was sometimes applied for 1 to 5 seconds with a set-up similar to that just mentioned. For other observations it was found more expedient to use brief pulses applied repetitively. A synchronous motor drove a cardboard circular disk at the rate of 30 revolutions per second. An angular slit with an amplitude of 60 to 180° cut in the disk allowed the intermittent illumination of a photoelectric cell for 5.56 to 16.67 msec., 30 times per second. A careful alignment of the borders of the slit in the disk with those of another slit 1 mm. wide in front of the photoelectric cell tended to render the make and the break of the discharges from the cell quite sudden. The current was made to full value or broken within an interval not greater than 0.2 msec.

The pulses from the photoelectric cell were intensified by one stage of d.c. amplification. A potentiometer regulated the output to the nerve. By changing the balance of the amplifier it was possible to deliver to the nerve small currents in either direction in the intervals between the pulses originated at the photoelectric cell. The use of this mode of stimulation was invaluable for the detailed analysis of the responses of the nerve to d.c. pulses. The sweep circuit of the oscillograph was driven 30 or 60 times per second from the a.c. main, which also drove the motor of the stimulator. An accurate synchronization could thus be obtained and the steady pattern on the face of the tube could be photographed or else the changes brought about by small changes of the voltage of the d.c. pulses could be readily appreciated and evaluated.

RESULTS. A. *The responses of nerve to ascending or descending currents of various intensities.* The observations were made mainly by stimulating through two electrodes on the popliteal nerve and recording the corresponding muscular mechanical responses. A current was delivered for 1 to 15 seconds and the same current was applied 1 to 5 minutes later through the same electrodes in the reversed direction. The effects of a given current were quite repeatable—that is, the responses to a given current were similar whether the application was made early in the experiment or later, after several other stimuli had been applied, provided that very strong currents were not used in the course of the observations.

When series of applications were made with increasingly higher voltages the results were as follows. Threshold was usually the same for both the ascending and the descending currents, provided the region of the nerve tested was sufficiently far away (about 3 cm.) from the cut end. Any difference present was entirely random if several electrodes were tested successively. That is, if the ascending current had a lower threshold than

the descending current with a certain pair of electrodes, changing one or both of these electrodes could lead to the opposite difference, and no evidence was encountered of any consistent effect anywhere along the nerve. The differences, when present, are therefore readily explained by unavoidable dissimilarities in the dissection of the nerve and in the intimacy of the contact of the several electrodes with the nerve.

The responses at threshold were invariably to the make of the currents, indicating that the break is a less effective stimulus than the make (confirming Pflüger, 1859).

As the currents were intensified, and usually before any contractions were obvious to the break of the currents, the responses to the make, which were single twitches at threshold, became tetanic—i.e., higher than a maximal twitch—thus showing repetitive firing by the nerve. In every one of the 43 nerves studied in these conditions such repetitive discharges were seen with quite weak currents (1.5 to 3 times rheobase).

Further intensification of the currents began to reveal marked differences between the effects of descending (i.e., cathode toward the muscle) and ascending (i.e., anode toward muscle) currents. First the tetanus corresponding to the make of the current (Pflüger's tetanus) was greater when the cathode was toward the muscle than in the reverse direction. The difference in the response to the make was due both to an increase for the descending and a decrease for the ascending current, as compared with the effects of weaker currents. Correspondingly, the tetanus at the opening of the current (Ritter's tetanus) was slightly greater when the anode was toward the muscle. With even stronger currents, the tetanus during the passage of the current was greater when the anode was proximal to the muscle than in the reversed direction. The responses to the make of the descending current were at this stage much smaller than previously, while the effects of the ascending application had increased considerably. Conversely, with these strong currents the opening tetanus was greater for the descending than for the ascending direction.

Figure 1 illustrates some of the characteristic steps in a typical series of observations, and the sequence of responses is summarized in table 1. It is important to note that the decrease of the responses to the make of the current when the cathode was toward the muscle (cf. fig. 1B, C, D) was probably not due to block of nerve impulses. Tests were made for block during the passage of the current, by stimulating supramaximally through electrodes placed further away from the muscles than those for the d.c. Only exceptionally was any significant block detected with the intensities of current discussed thus far. Absence of block or only slight partial block with characteristic responses to relatively strong d.c. are illustrated in figure 2.

In some experiments the nerve action potentials were recorded diphas-

ically during the passage of d.c. by means of electrodes placed peripherally. The nerve electrograms were closely parallel to the muscle mechanograms: large contractions were attended by rich bursts of spike potentials, whereas small contractions showed only discrete nerve impulses.

Table 1 differs significantly from Pflüger's (loc. cit.) classical "law of contraction" in two respects. First, the term "twitch," which was used throughout by Pflüger in summarizing his observations, is often substituted by the term "tetanus" in recognition of the fact, emphasized by Pflüger himself, that the responses of nerves to direct currents of intensities

Fig. 1

Fig. 2
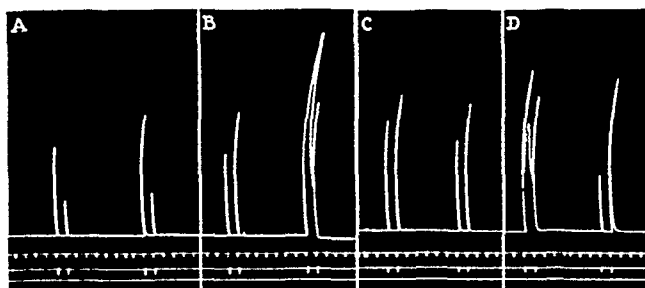
Fig. 1. Responses of the gastrocnemius-plantaris-soleus muscles to stimulation of the popliteal nerves with various intensities of direct current. Cat under dial. The electrodes were placed toward the middle of the thigh, with an interelectrodal distance of 15 mm.   The pairs of signals below the time mark indicate the beginning and end of a period of application of direct current (the same convention was followed in figs. 2, 3 and 4).   The make and break responses are arranged in pairs, the direct current having been applied for each voltage first in the ascending direction (anode toward muscle) and then in the descending direction (cathode toward muscle).

The voltages for the successive pairs were: A, 0.5; B, 2.0; C, 5.0; D, 10.0.

In this and the following kymograph records the time signal denotes 5-sec. intervals.

Fig. 2. Test for block of nerve impulses during responses to d.c. of relatively high voltage. Decapitate cat. The current (8 v.) was applied to electrodes placed low in the thigh with an interelectrodal distance of 8 mm., first with an ascending and later with a descending direction (pairs of signals, bottom line). Condenser shocks of an intensity 5 times maximal were delivered regularly, as shown by the twitches, to another pair of electrodes near the cut end of the nerve at the hip.
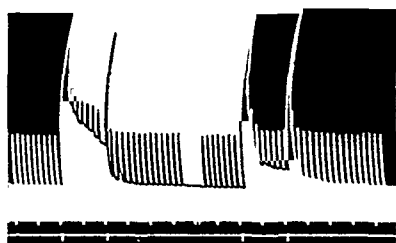
slightly greater than threshold are as a rule not single, but repetitive. Second, the effects attributed to strong currents are different from those reported by Pflüger. A probable explanation of this discrepancy, apart from the fact that Pflüger's statements refer to frog's nerves, may be that he worked exclusively with excised, that is non-circulated, nerves, whereas the present data apply to circulated nerves, in normal condition. In the excised nerves studied here the classical effects were the rule—that is, abundant repetitive discharges to the make or break of strong currents, according to whether the cathode or the anode of the d.c. was placed toward

the recording electrodes. That this difference in responses was due to the exposure and lack of circulation and not to the nature or mode of application of the electrodes was shown by the two following observations. In some cases the cut end of the nerve was freed up to one of the blood vessels supplying the sciatic, and electrodes shielded by glass tubing, of the Sherrington type, were applied to the dissected region. The results were similar to those in table 1, although it was necessary to employ higher voltages than usual. In other experiments the blood supply to the nerve was destroyed by crushing or tying all the visible vessels. The nerve was then left in its normal position and electrodes were applied to it as usually. The results were similar to those seen in the excised nerves— that is, similar to those described by Pflüger.

B. *Responses to stimulation through electrodes, one localized and the other diffuse.* In these experiments several silver chlorided electrodes were placed on the popliteal nerve, as usual, but the dry cotton pledgets em-

TABLE 1

Muscular responses to stimulation of circulated motor nerves by direct currents through two localized electrodes

CURRENT INTENSITY	ASCENDING DIRECTION		DESCENDING DIRECTION	
	Closure	Opening	Closure	Opening
Weak..........	Twitch		Twitch	
Moderate......	Medium tetanus	Twitch	Medium tetanus	Twitch
Strong.........	Small tetanus	Medium tetanus	Large tetanus	Small tetanus
Very strong...	Large tetanus	Small tetanus	Small tetanus	Large tetanus

ployed in other cases to isolate the nerve as much as possible from surrounding tissues were avoided. A heavy chlorided silver needle was further inserted subcutaneously or intramuscularly in the middle of the thigh. The d.c. was then delivered, using this needle as one of the electrodes— the diffuse one with respect to the nerve—and any of the wires in close contact with the nerve as a localized electrode where the current density was heaviest.

The tests were made as in the previous section, by applying d.c. of various intensities for 1 to 10 seconds, making the localized electrode alternatively anode or cathode. With progressively increasing voltages the first response detected, a twitch, corresponded invariably to the make of the current when the localized electrode was the cathode. This response grew thereafter, promptly becoming tetanic in character, and only decreased later, with fairly strong voltages (15 to 30 times rheobase).

The next response to appear was also at the closure of the current, when

the anode was localized. This response in turn increased with greater voltages, and kept on augmenting when the response to the make with a localized cathode had already begun to decrease. It was always possible to evoke with a sufficiently high voltage a greater response to the make of the current with a localized anode than with a localized cathode (figs. 3 and 4).

The responses to the break of the currents appeared exceptionally with weaker voltages when the anode was the localized electrode. The converse was, however, usually seen. The response to the break with a localized
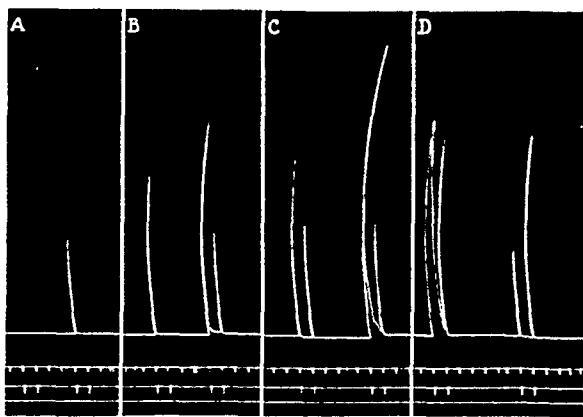
Fig. 3   Fig. 4

Fig. 3. Muscular responses to stimulation of the popliteal nerve with electrodes, one localized (needle in contact with the nerve) and the other diffuse (needle in the subcutaneous tissue). Cat under dial. As in figure 1, the responses are arranged in pairs, the currents having been delivered with the localized electrode first as the anode and then as the cathode. The voltages for the successive pairs were: A, 1; B, 2; C, 4; D, 8.
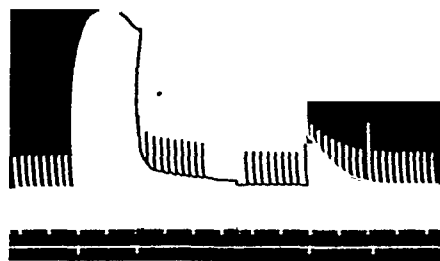
Fig. 4. Test for block of nerve impulses during responses to d.c. of relatively high voltage. Decapitate cat. The current (10 v.) was applied through one localized electrode placed low in the thigh and another diffuse electrode as in figure 3. The localized electrode was first the anode and then the cathode. Supramaximal condenser discharges were delivered regularly, as shown by the twitches, through another pair of electrodes near the hip.

cathode could be present with currents much weaker (up to $\frac{1}{10}$) than were necessary when the anode was the localized electrode (table 2, fig. 3).

With strong currents the contractions were typically as follows. If the cathode was the localized electrode the response to the make was small, that to the break large. Conversely, with a localized anode the response to the make was large, that to the break relatively small (figs. 3 and 4).

Tests for block during or after the applications of d.c. were made by stimulating with condenser discharges of an intensity 5 to 10 times maximal a region of the nerve 3 to 6 cm. central to the point where the localized electrode was placed. The intensities of d.c. required to produce block

were greater than those which had resulted in a relative decrease of the responses to the make with a localized cathode (fig. 4). In figure 4 the localized anode elicited a practically maximal tetanic contraction. It is not possible to judge, therefore, whether or not block was present in that observation. Experiments in which the tetanic responses to d.c. were less marked than those in figure 4, however, showed that a localized cathode produced invariably block at lower voltages than did a localized anode (cf. Bishop and Erlanger, 1926).

In a few experiments in which the diffuse electrode was obtained by connecting together several electrodes placed on the nerve either centrally, or peripherally, or both, with respect to the localized single electrode, the results were quite similar to those seen with the needle in the subcutaneous tissues.

C. *Responses to brief d.c. pulses delivered repeatedly at short intervals.* As explained under Method, such brief (5.6 to 16.7 msec.) pulses were

TABLE 2

Muscular responses to stimulation of circulated motor nerves by direct currents through electrodes making one a localized and the other a diffuse contact

CURRENT INTENSITY	LOCALIZED CATHODE		LOCALIZED ANODE	
	Closure	Opening	Closure	Opening
Weak..........	Twitch			
Moderate......	Small tetanus		Twitch	
Strong.........	Large tetanus	Twitch	Small tetanus	
Very strong...	Small tetanus	Large tetanus	Large tetanus	Small tetanus

obtained by the regular illumination of a photoelectric cell at the rate of 30 per second. The observations were all made on excised nerves, the spike potentials of the A fibers serving as indicators of nerve activity.

In a series of observations the stimuli were delivered to one end of the nerve and the responses were recorded monophasically from the other end. The quite regular responses which resulted from stimulation by the d.c. pulses at various intensities were photographed with exposures of $\frac{1}{5}$ second during the course of stimulation. The regularity of the responses is emphasized by the fact that, although several sweeps (5 or 6) of the spot traveled over the face of the cathode-ray oscillograph during the exposures, the lines appear single on the records (figs. 5, 11 and 12).

Certain features of the responses obtained in these conditions will be described below in connection with the repetitiveness of nerves stimulated by d.c. For the present, the following important characteristics will be emphasized. With the stimulating electrodes placed one on a crushed and the other on an intact region of the nerve, the latency of the responses

to the make or break of the d.c. pulses could be made approximately the same, by proper adjustment of the voltage, whether the anode or the cathode was on the undamaged nerve. This was true even when the distance between the two stimulating electrodes was relatively long, e.g., 7.5 cm.

When the stimulating electrodes were both on intact regions of the nerve the response to the break of the d.c. pulses was often double—i.e., two submaximal volleys with different latencies. The magnitude of the two components changed independently when the intensity of the stimuli was varied. The phenomenon is illustrated in figure 5.

In another series of experiments the record was obtained from a region of the nerve placed between the stimulating electrodes. The purpose of these observations was to determine by the polarity of the diphasic
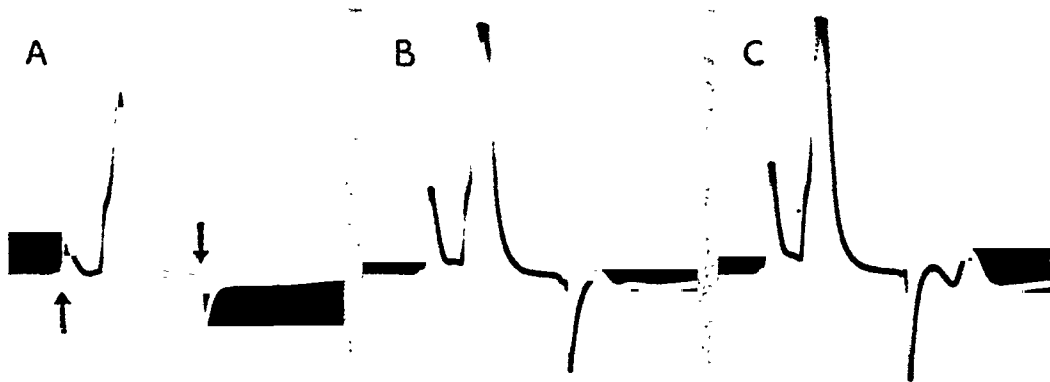
Fig. 5. Double responses to the break of d.c. pulses (5.6 msec.). The record was taken monophasically from the peripheral end of the peroneal nerve. The stimulating electrodes were placed, the anode 6.6 and the cathode 7.6 cm. away from the recording leads. Both stimulating electrodes were on intact regions of the nerve. In A no response to the break of the pulse is seen (the arrows indicate the artifacts of the make and break of the pulses). In B a response with a short latency is visible. In C an additional response appears with longer latency. Voltages (conventional units): A, 4; B, 5; C, 5.5.

response thus obtained the direction from which the nerve impulse reached the recording electrodes—i.e., the pole of the stimulus at or near which the corresponding impulses originated. For if an impulse conducted from b toward e in figure 6 should appear in the record as an "up"—then "down" excursion, an impulse traveling from e to b would record as a "down"-"up" diphasic complex.

Certain precautions are necessary for these records. The main technical problem encountered was to reduce the large d.c. artifact to sufficiently small or brief dimensions so that the response would be clearly recognizable and its polarity identified. The set-up shown in figure 6 was found satisfactory. The small capacities (C_2) placed beyond the recording electrodes effectively reduced the d.c. artifact. They further served the

purpose of eliminating a spurious anode or cathode at the points c and d, since the d.c. could not flow through such capacities. The time constant corresponding to such capacities and to the 50,000 ω potentiometer from which the Wagner ground was led, was adequate for the recording of the spike potentials without serious distortion. The Wagner ground, with or without capacity and resistance, further reduced the artifacts and was of great value, when shifted during a response, for recognizing a doubtful polarity. As may be readily understood, the results were not always clear. For if impulses should arrive at the recording electrodes from both ends of the nerve simultaneously or at close intervals the responses might collide if coming over the same fibers, or they might at least partially cancel for recording purposes even when coming over different fibers of the nerve.

Certain clear effects were encountered, however, as follows. In figure 7 are illustrated records of a typical experiment from a fresh (recently excised) nerve from a decapitate (unanesthetized) preparation. The stimulating electrodes were placed one on an intact region 3 cm. away from the peripheral cut of the peroneal nerve and the other on the crushed central end. The electrode on the intact region was first made the anode for the brief (5.6 msec.) d.c. pulses. As the voltage was progressively increased the responses to the break of the pulses appeared with the polarity corresponding to impulses set up from the region of the nerve where the anode lay. Stronger pulses led to the appearance of a response to the make of the pulses, and their polarity was similar to that of the responses to the break—i.e., they originated from the region of the nerve where the anode was placed, not from that to which the cathode was applied.

When the polarity of the d.c. pulses was reversed, that is, when the anode was in contact with a crushed and the cathode with an intact region of nerve, the first responses to appear were to the make of the current, and their polarity indicated that they came from the region of the cathode. Further intensification of the current led to the appearance of a response to the break of the pulses. This response had again the polarity corresponding to origin at the cathode, not the anode of the stimuli.

As already emphasized, this effect was commonly encountered in the fresh nerves from spinal animals. It was only rarely seen in nerves excised from animals anesthetized with dial, or in nerves which had been excised and observed for 1 to 3 hours.

Another characteristic effect seen in about one-half of the nerves studied was the following. With the stimulating electrodes placed as before, one on intact, the other on crushed nerve, a response to the make or break of the d.c. pulses appeared with a given intensity of current. Further intensification of the stimuli led then to the appearance of an additional response which preceded or succeeded the first one recorded. The polarity
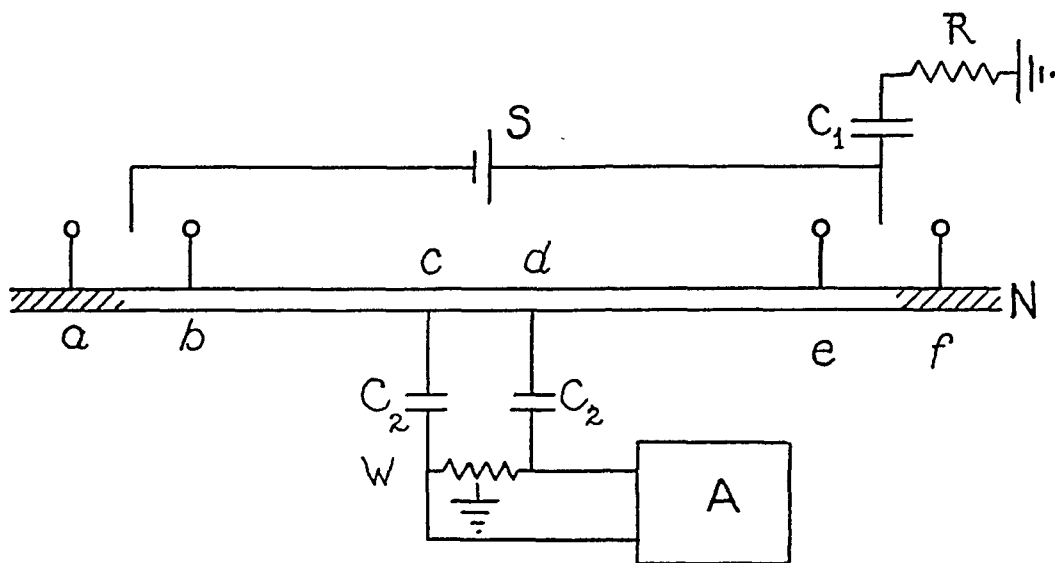
Fig. 6. Diagram of the arrangement used for recording the nerve action potential diphasically between the two d.c. poles. The letters mean: S, d.c. stimulator; R, 10,000 to 100,000ω; C_1, 0.1 μF; N, nerve, the shaded areas represent the crushed ends; a to f, electrodes; C_2, 0.001 μF; W, Wagner balance to ground through a 50,000ω potentiometer; A, amplifier.
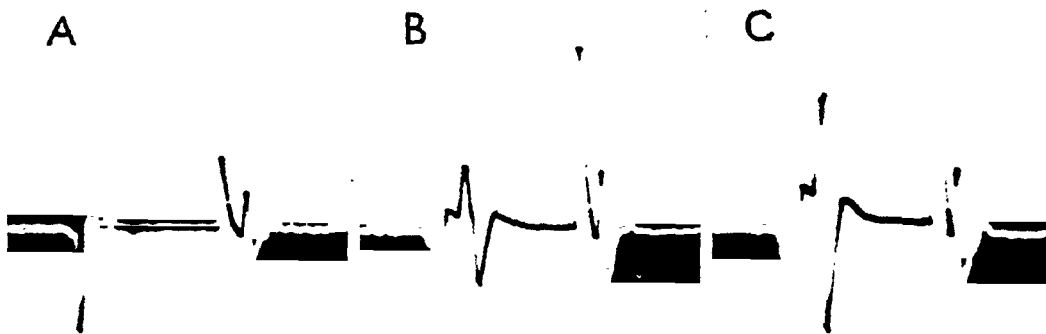
Fig. 7. Responses from the anode at the closure and opening of d.c. Excised peroneal from a spinal animal. The experimental set-up was as shown in figure 6, with the anode of the d.c. at b (intact nerve) and the cathode at f (crushed nerve). The monophasic artifacts show the beginning and end of the pulses (5.6 msec.) delivered 30 times per sec. In A only a response to the opening of the current is present; the polarity is that which corresponded to impulses traveling from b to f. In B a response to the make of the pulses appears; its polarity is similar to that of the response to the break of the pulses (as in A). In C, with a higher voltage, the response to the make is greatly increased. Voltages (in conventional units): A, 3; B, 4; C, 5.

of this additional response was opposite to that of the original one, thus showing that at either the make or the break, or both, nerve impulses were being originated, probably over different fibers, from both poles of the stimuli. Typical instances of this effect are illustrated in figure 8.

In all the observations described in this section the responses attributed to one of the poles showed a decrease or an increase of latency when the recording electrodes were moved closer to or further from that pole. In
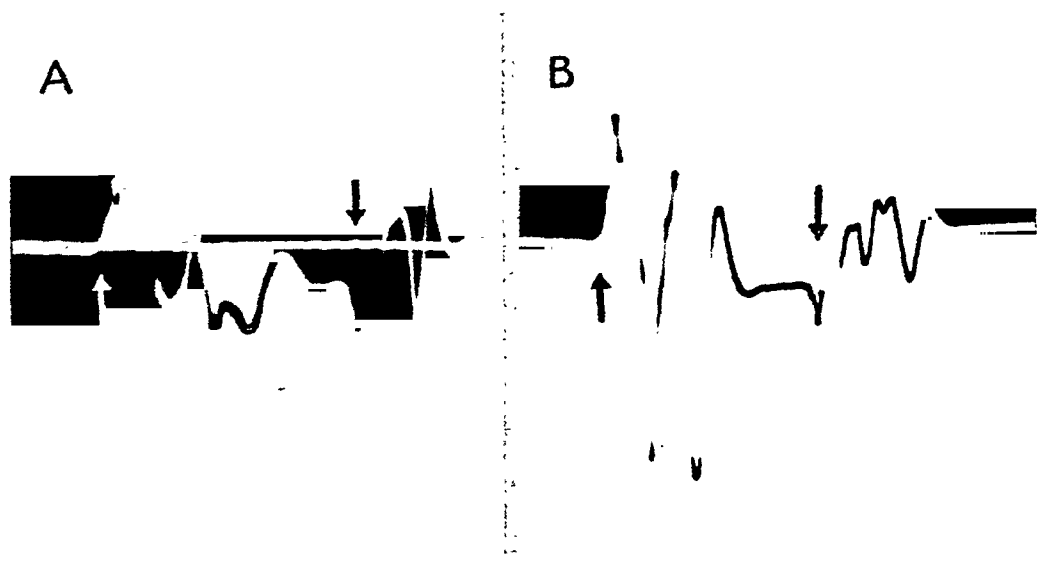
Fig. 8. Double responses of opposite polarity to make and break of the d.c. pulses (5.6 msec.). The experimental set-up was as shown in figure 6. Peroneal nerve. The arrows indicate the make and break artifacts.

In A the cathode was at a (crushed nerve) and the anode at e (intact nerve). The single response to the break of the pulses shows the polarity of the record of impulses traveling from e to a. The response to the make is double; a submaximal volley going from a toward e is followed by a volley traveling in the opposite direction.

In B the d.c. electrodes were as in A, with the anode on an intact and the cathode on a crushed region of the nerve. The recording electrodes c and d were closer to the anode than to the cathode of the d.c. pulses. The responses to both the make and the break are double; a volley from the anode is followed by a volley from some point near the cathode.

the cases of double response of opposite polarity (fig. 8) it was sometimes possible by continuous application of d.c. from another source near one of the stimulating poles to increase or decrease selectively one or the other of the two diphasic components of the complex response. A similar relative separation of these components was also obtainable by sending through the stimulating electrodes d.c. in either direction in the intervals between the stimulating pulses. As explained under Method, such additional currents could be readily obtained by changing the balance of the amplifier in the output of the photoelectric cell.

D. *The repetitive nature of the responses of nerve to continuous applications of d.c.* As already emphasized (pp. 102, 104, figs. 1 and 3), the muscular responses to d.c. were invariably tetanic when the intensity was 1.5 to 3 times that of the threshold of the most excitable motor fibers in the nerve. Slightly more intense currents led usually to the appearance of large tetani which could be well sustained for periods of over 10 seconds (fig. 4).

The conditions which favored the appearance of well-sustained repetitive responses may be summarized by the general statement that the more fresh and normal the nerve the greater the ease with which such responses were elicited. Thus, in all the experiments made on spinal animals after the ether given during anesthesia had been eliminated, well-sustained tetani could be readily elicited with weak currents. In many of the animals studied under dial anesthesia, on the other hand, although tetanic responses could always be evoked, they were only poorly sustained, even at relatively high voltages.

The presence or absence of the circulation in the nerves was also found significant. Well circulated nerves were more apt to show repetitive discharges than were nerves whose circulation had been deliberately or unwittingly damaged. Similarly, nerves *in situ* showed invariably richer repetition than did the same nerves or others from the same animals when observed even immediately after excision.

Regions of a given nerve which had been stimulated for some time yielded less repetition, even after prolonged rest, than that resulting from similar currents applied to other relatively fresh regions of the same nerve. Finally, in the excised nerves the probability of eliciting repetitive responses decreased progressively with time.

The repetitive nature of the responses to d.c. was seen regularly not only upon the closure of the currents, but also when currents of sufficient intensity were opened. Higher voltages were necessary, however, for the appearance of opening tetani than those sufficient for the production of the closing tetani. Stimulation by prolonged applications of d.c. to nerves *in situ* or excised resulted usually in quite irregular electrograms (fig. 9). Not infrequently, however, the responses of the different fibers tended to synchronize, causing then the appearance of fairly regular waves in the electrical records. In figure 10 is illustrated one of the outstanding instances of such synchronization. The records were taken at 0.5-second intervals.

The method of stimulation and recording described on p. 106 was found very satisfactory for the detailed study of the repetitive responses both to the make and the break of the current. The photoelectric cell was illuminated 30 times per second for 11.1 or 16.7 msec., the intervals between the stimuli being, therefore, 22.2 and 16.7 msec., respectively. Whether both or only one of the stimulating electrodes was in contact with intact nerve, and whatever the polarity of the stimuli with respect to the

recording electrodes, it was always possible to record repetitive responses both to the on and the off of the stimuli if the voltage was raised sufficiently.

Two interesting features were regularly seen. First, the frequency of the repetitive discharges was directly related to the intensity of the pulses delivered. Thus, by progressively increasing the voltage during a period of stimulation, when threshold was attained a response to the closure appeared. Slight intensification resulted in the growth of this first response and in the appearance of a second response late during the period of passage of the current. This second response then increased in magnitude and decreased in latency until a third response appeared. A similar process could recur until it was possible to see a regular pattern of as many as 7 waves occurring during the 16.7 msec. that the current was applied. A series of pictures illustrating the phenomenon is shown in figure 11. These pictures give only a suggestion of the thoroughly continuous and quite systematic changes seen during the gradual increase or decrease of intensity.
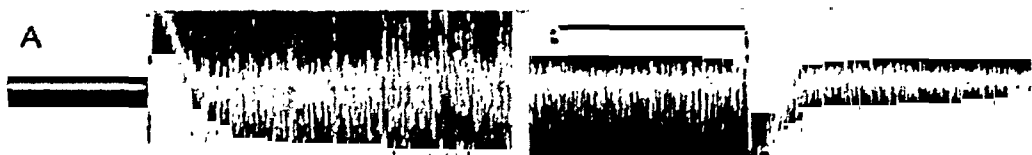
Fig. 9. Irregular responses of nerve to a prolonged application of d.c. Excised peroneal. Cathode of d.c. toward recording leads. A, closure of d.c., 2 v. B, opening of d.c., after 5-sec. application.

The responses to the break of the pulses showed likewise characteristic repetition, with frequency again proportional to intensity (fig. 12). Higher voltages of stimulation were necessary to see this effect than were sufficient to elicit marked repetition during the period of passage of the pulses. The greatest frequency recorded (7 waves during 22 msec.) was lower than those which could be readily obtained during the "on" period.

The second feature of the repetitive effects worthy of emphasis was the regularity of the responses registered. In some instances the patterns of repetition were quite complex, due probably to the fact that different groups of fibers were discharging at different frequencies and due probably, also, to simultaneous stimulation at the cathode and the anode. Yet such complex patterns could be sustained so well that pictures taken up to 1 minute apart showed only insignificant differences (cf. fig. 12, F and G).

DISCUSSION. The classical concept, that stimulation occurs only at the cathode upon closing and only at the anode upon opening a d.c. applied to nerve, fails to account for some of the data described in sections A and B. Thus, the increase of the closing tetanus for the ascending current,
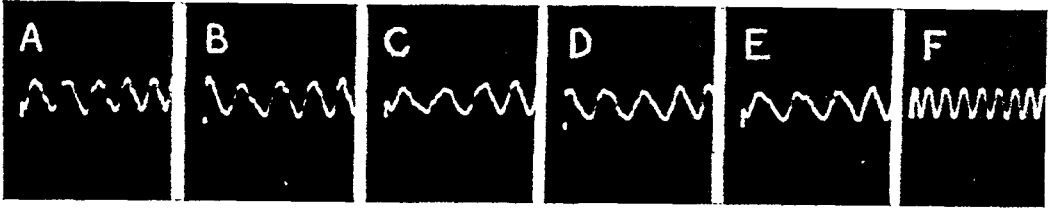
Fig. 10. Synchronized repetitive responses of nerve to a prolonged application of d.c. Excised peroneal nerve from a spinal cat. Cathode of d.c. toward the recording leads. The successive pictures, A to E, were taken at half-second intervals beginning immediately after the application of d.c. The amplitude of the synchronized waves is about 20 per cent of a maximal A spike. F, 1,000 cycles.
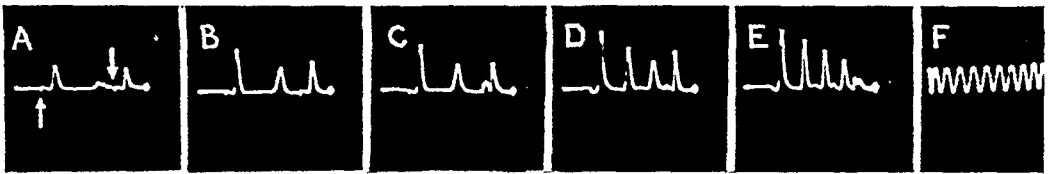
Fig. 11. Repetitive responses of nerve during the passage of d.c. Brief (11.1 msec.) pulses applied at the rate of 30 per sec. to an excised phrenic nerve from a spinal cat. The on and off artifacts of the pulses appear as small diphasic excursions (arrows in A). The cathode of the stimuli was proximal to the recording leads. The conduction distance was 6 cm. Voltages (in conventional units): A, 5; B, 6; C, 7; D, 8; E, 9. F, 500 cycles.
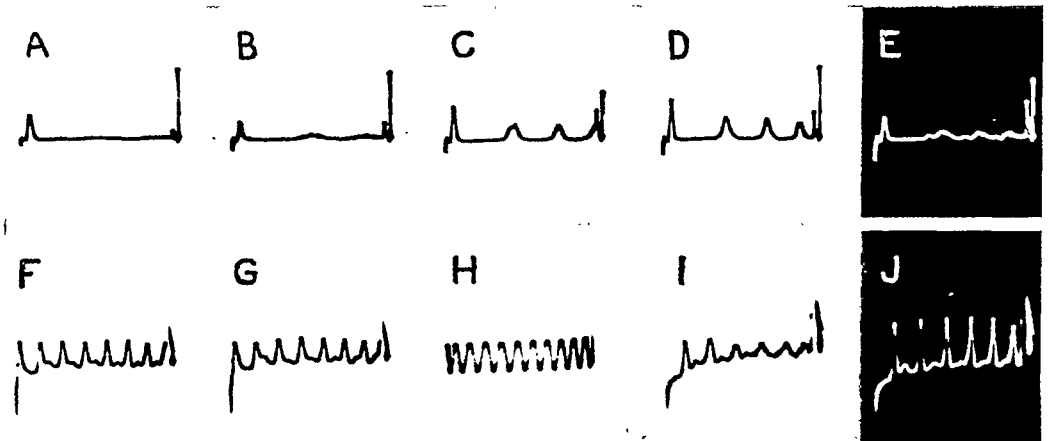
Fig. 12. Repetitive responses of nerve after the passage of d.c. Brief (11.1 msec.) pulses applied as in figure 6. The records begin with the artifact showing the break of the pulses and end with the artifact for the make of the pulses and the corresponding responses (crowded together at the end of the sweep). In records A to G the anode of the stimuli was proximal to the recording leads; in records I and J the polarity of the stimuli was reversed. Voltages (in conventional units): A, 6; B, 7; C, 8; D, 10; E, 12; F and G, 25; I, 22; J, 35. Record G was taken 1 min. after F, the nerve being stimulated continuously at a constant voltage. H, 500 cycles.

with a corresponding decrease of the response to the closure of the descending current as the voltage is intensified (fig. 1) is not in accord with that concept. The opposite effect seen in similar conditions for the responses to the opening of the currents (fig. 1) is likewise not explainable on that basis.

Pflüger (1859) attributed the difference of effects of strong ascending or descending currents to a block of the impulses set up at the pole distal to the muscle by the deleterious blocking effects of the proximal pole. It is interesting to note, however, that he did not test experimentally for this block. When such tests were made in the present study it was frequently found (fig. 2) that block of impulses set up centrally to the d.c. electrodes was negligible or absent.

The failure of a strong descending current to cause as large a muscular response as that elicited by the same, but ascending current (fig. 2) might be considered as due to Wedensky inhibition, because of too high a frequency of impulses reaching the muscle when the cathode is proximal. That this is not the case was shown by the fact that single maximal twitches could be elicited during the period of application of the current. Indeed, that the muscle was in all instances a faithful indicator of activity in the nerves was shown by the close parallelism found when the mechanograms were compared to the electrograms recorded from a region of the nerve between the d.c. electrodes and the muscle (p. 103).

From the responses in figure 2, it is clear, therefore, that the large tetanus produced by the closure of the ascending current is not due to impulses starting at the cathode. If such were the case the descending current should produce as large or a greater effect, but it does not. The conclusion appears reasonable that the impulses originate at the anode and that they are conducted in one direction only, away from the d.c. poles. An apparent paradox is thus revealed, in that whereas these impulses would be blocked toward the cathode, impulses coming from higher regions of the nerve are not blocked. The discrepancy may be solved in one of two ways. First, the observations of Hodgkin (1938) suggest that nerve impulses do not start directly as consequences of electric stimuli, but that they are preceded by a local, unpropagated response which, if sufficiently great, initiates the conducted disturbance. It is conceivable, therefore, that in figure 2 the anode sets up local responses which succeed in provoking the appearance of conducted waves only in the direction opposite the cathode. The full-sized nerve impulses reaching the anode would, on the other hand, be conducted through without block.

A second explanation could be that there is a polarity in motor nerves such that the direction of the d.c. is significant not only with respect to the muscle, but also in relation to the stimulating ability of the current. In other words, it is possible that a strong ascending current is a better stim-

ulus for a motor nerve than a descending current. This possibility is, however, rendered unlikely by the absence of any evidence of polarity in nerve fibers when the influence of d.c. on their electrical excitability is analyzed (Rosenblueth, 1941).

With the problem of one-way stimulation dismissed, the data in figure 1 and in table 1 suggest that both the anode and the cathode can stimulate both at the make and at the break of d.c. The make of the cathode appears to increase in efficiency with increasing voltage up to an optimum and then to decrease for stronger voltages. The make of the anode does not show this decrease with strong currents. The break of the anode seems to behave like the make of the cathode, while the break of the cathode behaves like the make of the anode, as the intensity of the currents is increased.

Results obtained by "unipolar" methods of stimulation were declared by Biedermann (1895) unsuitable for accurate interpretation. He pointed out that the term was inadequate; if there is a cathode in a nerve there must also be an anode, and *vice versa*. In recognition of this legitimate criticism the term unipolar has been avoided in this report and the expression localized, as opposed to diffuse, has been substituted. Biedermann concluded that it is impossible to estimate the rôle of uncontrolled spurious poles, that is, unknown regions where the current enters or leaves the nerves.

The possibility of spurious poles should, of course, be kept in mind in evaluating the experiments described in section B. Thus, the responses to the closure of weak currents with a localized anode (fig. 3B) might be attributed to spurious cathodes. With the experimental conditions adopted, however, this assumption appears unreasonable. The cathode was spread by the tissues to a quite large surface of the nerve. The points where the current would leave the nerve with greater density should be those in the close vicinity of the localized anode, that is, those where, according to classical theory, the electrical excitability should be maximally depressed. It is probable, therefore, that these responses were due to the anode and not to a hypothetical concentrated cathode.

The fact that the responses to the opening of the currents occurred usually with lower voltages when the cathode rather than the anode was localized (fig. 3) is incompatible with the classical theory. A break response, with a localized cathode, would be due to a spurious anode. Why, then, should not the certainly more concentrated effect of the localized anode yield an even greater response? On the other hand, all the results in section B (figs. 3 and 4, and table 2) fit the theory expressed above as suggested by the analysis of the results in section A.

Several authors (see Mares, 1913; Thörner, 1923; Woronzow, 1924) have come previously to the conclusion that the classical theory of nerve

stimulation by d.c. is incomplete.   Their results will not be discussed here.
Suffice it to say that in their experiments, like in those in sections A and B,
the indicator of nerve activation was the muscular mechanical response.
It might be objected that such observations furnish only indirect evidence
with regard to problems of nerve function.   That objection does not apply,
however, to the results in section C.

Inferences on the stimulating action of d.c. based on measurements of
latency are complicated by the influence of d.c. on conduction velocity.
Because of this complication the latency of a response was used only
sparingly to determine its site of origin in the nerve (p. 107).   The polarity
of a diphasic record, on the other hand, is a certain indicator of the region
of the nerve from which an impulse reaches the recording electrodes.   The
observations in figures 7 and 8 prove, therefore, that both the anode and
the cathode may stimulate nerves both at the closure and at the opening
of d.c.   The records in figure 8 show further that it is possible to obtain
simultaneously anodal and cathodal excitation at either the make or the
break of d.c.   This explanation accounts satisfactorily for the complex
responses to the break of the d.c. pulses in figure 5.   The two components
of these responses correspond to impulses set up by the anode and cathode,
respectively, over different fibers.

The problem of the repetitiveness of nerve responses to continuous
stimulation by d.c. has been only little studied thus far.   Indeed, although,
as already mentioned, Pflüger (1859) emphasized that tetani, instead of
twitches, were the usual muscular responses in fresh preparations, and
although this fact has been abundantly confirmed since (see figs. 1 to 4
and 9 to 12), text-books still describe twitches as the normal responses to
d.c. and only mention tetani as abnormal effects of very strong currents.

In experiments on the excised dog's phrenic nerve Erlanger and Blair
(1936) found intense spontaneous activity.   Both single shocks and
rectangular currents elicited repetitive discharges when applied to a region
of the nerve with the sheath intact.   If the sheath was removed the
repetitive responses disappeared.   These results differ from the present
observations.   The nerves studied showed little or no spontaneous activity,
except when deteriorated by exposure or experimental handling.   Repeti-
tive responses could invariably be elicited by d.c., although the sheath
(only slight in the cat as compared to the dog) was always removed as
much as possible during dissection.   The main difference in the experi-
mental conditions appears to be that Erlanger and Blair observed the
phrenic nerves only after they had been placed in Locke's solution in a
refrigerator for several hours, whereas in these experiments the observa-
tions were made as soon as possible after excision.

Erlanger and Blair (loc. cit.) found that anodal polarization favors the
appearance of repetitive responses.   On this basis they suggest that the

repetition which has been observed in intact human nerves (Ebbecke, 1924) may be due to a normal state of anodal polarization descending to the nerves from the centers (subordination; Lapicque, 1923). Such an explanation cannot apply to the present results. Repetition always occurred after the nerves were cut. Indeed, in a few observations made to test the point, no differences in the muscular responses to d.c. were seen in records taken before and after cutting the sciatic nerve.

The results illustrated in figures 9 to 12 lead to the following conclusions. Repetitive discharges are the normal response of nerve fibers to continuous applications of d.c. of voltage slightly greater than threshold. The fresher and more normal the nerve, the greater its ability to exhibit repetition (p. 111). Although the rates at which individual fibers repeat in response to a given treatment with d.c. may vary considerably, the integrated effect on a large number of fibers shows statistical uniformities—i.e., a large majority of the A fibers in a nerve tend to repeat at the same rate, especially if stimulated by brief d.c. pulses. The number of fibers sharing in the successive responses during repetition and the frequency of the repetition increase with the voltage of the d.c. applied. The preceding statements apply both to the closure and to the opening of d.c.

The changes of the electrical excitability of nerve produced by applications of d.c. are described and discussed in a separate report (Rosenblueth, 1941). It may be mentioned here that those changes are in agreement with the conclusions inferred in the present study. Thus, if it is possible to obtain stimulation by the make at the anode and by the break at the cathode, it should be possible to demonstrate increases of excitability in these conditions with subthreshold currents. This was found to be the case.

It is hardly necessary to point out that the results reported in this study and the inferences derived therefrom entail a serious revision of current theories of nerve function. These theories are based on simplified premises drawn from the classical experiments, made almost exclusively on excised frogs' nerves. For example, as mentioned before, although it has been repeatedly shown that the common response of normal nerves to d.c. is not single but repetitive, yet emphasis is usually placed on the marked ability of nerve to "accommodate" to a continuous stimulus. Similarly, although Pflüger (1859) reported that the most prominent aftereffect of cathodal polarization is frequently an increased, instead of a decreased excitability, only the post-cathodal depression has been incorporated in the current concepts. The study of circulated or freshly excised mammalian nerves confirms all the positive classical findings and brings out additional data. It is likely that these new properties will also be exhibited by batrachian nerves. But even if such should not be the case a general theory of nerve should include all the data.

SUMMARY

The responses of circulated cat's motor nerves to ascending or descending direct currents (d.c.) of variable voltage (figs. 1 and 2; table 1) suggest that stimulation may occur not only at the cathode at make and at the anode at break, but also in the reversed relation.

The suggestion is supported by the results of stimulating through a localized electrode on the nerve and a diffuse lead through the surrounding tissues (figs. 3 and 4; table 2).

Observations made on excised nerves (figs. 5 to 8) give direct evidence that the suggestion is correct.

Applications of prolonged d.c. or of brief pulses result in repetitive responses from the nerves if the voltage is over 1.5 to 3 times rheobase (figs. 1 to 4 and 9 to 12). The frequency of this repetition increases with the voltage of the d.c. (figs. 11 and 12). These statements apply both to the closure and the opening of d.c., but repetition at the opening requires higher voltages than repetition during the passage of the current.

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THE STIMULATION OF MYELINATED AXONS BY NERVE IMPULSES IN ADJACENT MYELINATED AXONS

A. ROSENBLUETH

From the Department of Physiology in the Harvard Medical School, Boston, Mass.

The stimulation of nerve fibers by the activity of adjacent muscle elements is well known. The activation of non-myelinated nerve fibers by nerve impulses traveling over similar adjacent elements was occasionally seen by Jasper and Monnier (1938). Arvanitaki (1940) treated the region where two such fibers were in contact with sodium citrate, in order to increase the local excitability. In those conditions a nerve impulse in one of the fibers elicited regularly an impulse in the adjacent element, with a latency of less than 5 msec.

No observations of a similar phenomenon have been reported for myelinated fibers. Indeed, Blair and Erlanger (1932), on the basis of negative results, concluded that the nerve impulses in a myelinated fiber fail to produce even a subliminal excitation of the adjacent elements.

The present report describes conditions in which activation of myelinated mammalian axons will regularly occur when nerve impulses travel through the neighboring elements in the same nerve trunk.

METHOD. The nerves studied were mainly the peroneal and occasionally the popliteal and phrenic of the cat. The animals were either anesthetized with dial (Ciba, 0.75 cc. per kgm.) or decapitated under ether anesthesia. In the decapitate animals artificial respiration was administered and the anesthesia discontinued. One-half to four hours later the nerves were dissected and excised. They were then placed in a moist chamber for study.

The nerve action potentials were recorded monophasically or diphasically from a cathode-ray oscillograph, after adequate amplification.

The test stimuli were condenser discharges through a thyratron, rendered diphasic by means of a transformer. They were delivered at frequencies of 2 to 60 per sec. Direct currents (d.c.) were sent to the nerves through impolarizable calomel electrodes.

In some experiments the contractions of the tibialis anticus muscle were used as indicators of nerve impulses in the motor fibers of the peroneal nerve, cut centrally. The leg was then fixed by drills inserted into the tibia. The tendon of the muscle was attached to a tension myograph. Upward excursions in the kymograph records denote contraction.

RESULTS. Three pairs of electrodes were placed on the excised nerves, one for recording purposes, one for the test stimuli, and one for d.c. For convenience in referring to the position of the electrodes the following convention will be used: r_1 and r_2 will indicate the recording pair, s_1 and s_2 the pair used for the test stimuli, and c_1 and c_2 the pair of impolarizable electrodes used for d.c.

In a series of observations r_1 and r_2 were placed toward either end of the nerve, r_1 being on the crushed extremity. Next came s_1 and s_2; and then c_1 and c_2, both pairs on intact regions of nerve. In these conditions, if the stimuli were submaximal, a simple A spike was present in the records. Applications of d.c. through c_1 and c_2 resulted, of course, in changes in the amplitude of the original spike, due to changes of excitability and of the spike magnitude of the responding elements. But in addition to these
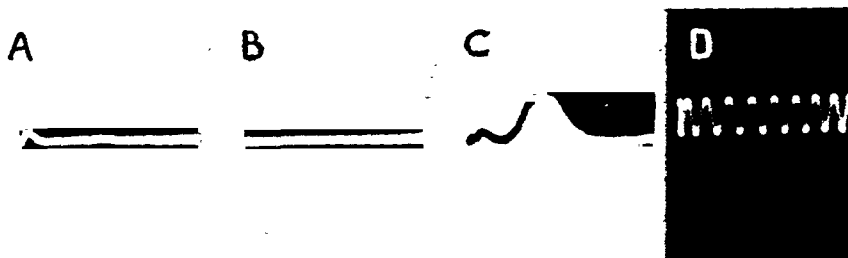
Fig. 1. Appearance of a late response after application of d.c. Monophasic record from a region 1.5 cm. away from the crushed central end of an excised peroneal nerve. Stimulating electrodes: anode 2.2 and cathode 3.0 cm.; and d.c. electrodes: anode 5.3 and cathode 7.0 cm. away from the same end of the nerve. Submaximal stimuli applied regularly at $\frac{1}{2}$-sec. intervals.

A. Response before application of d.c. B. Absence of response during the passage of d.c. (4 v.). C. Early and delayed responses 1 sec. after opening of the d.c. D. One thousand cycles.

well-known changes, the electrogram was frequently complicated by the appearance during or after the passage of d.c. of 1 or 2 later spike potential waves (figs. 1 to 5).

These late waves were spread out in time, that is, they showed considerably more temporal dispersion than the first early response. As a rule, the late spikes were smaller than the early response (figs. 2 and 3), but occasionally the amplitude of one or both of them could be larger than that of the first spike (figs. 1 and 4). If the intensity of the stimuli was too small (activation of only a few fibers) or too great (maximal A initial response) no delayed spikes could be detected with any intensity or polarity of d.c.

With a fixed position of the recording and stimulating electrodes the latency of the delayed spikes varied with the position of the d.c. electrodes on the nerve as follows. With relatively weak d.c. (about twice rheobase)

there was usually only one late spike during the passage of the current. The latency of this spike depended on the position of the cathode of the d.c. The greater the distance between this cathode and the stimulating electrodes, the longer the interval between the early and the late response.

With relatively weak d.c. only one late spike appeared after the current was broken. The latency of this wave depended on the position of the

Fig. 2. Appearance of late responses during and after applications of d.c. Monophasic record from a region 2.7 cm. away from the peripheral cut end of the peroneal nerve. Stimulating electrodes: cathode 4.3 and anode 5.6 cm.; and d.c. electrodes: cathode 7.1 and anode 8.6 cm. from the same end.

A. Early response to a submaximal test stimulus. B. During the application of d.c. (0.4 v. across the nerve). The late wave starts at the cathode. C. One second after opening the d.c. The late wave starts now at the anode, hence its longer latency. D. Calibration of sweep, 1,000 cycles.
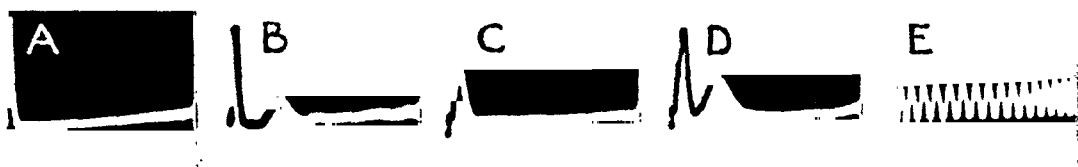
Fig. 3. Appearance of late responses after applications of d.c. Monophasic record from a region 1.5 cm. away from the central cut end of the peroneal nerve. Stimulating electrodes: cathode, 3.0 and anode 4.5 cm.; d.c. electrodes: 6 and 7.5 cm. from the same end.

A and B. During, and 2 sec. after the application of 0.2 v. d.c. with the anode distal to the recording end.

C and D. As in A and B, but with the anode of the d.c. proximal to the recording end. The latency of the late response is shorter in D than in B.

E. One thousand cycles.

anode of the d.c. (fig. 3). When with stronger currents two late waves were present the latency of each varied with the distance to the stimulating cathode of the anode and cathode of the d.c., respectively.

Changes in the interval of time separating the initial and any of the late responses could also be produced by keeping the position of the d.c. and recording electrodes constant while varying the site of application of the stimulating electrodes. The results were similar to the previous—i.e.,

with short distances between the stimulated region and the region treated by d.c., the late responses followed promptly the first spike, and conversely with long distances a marked delay separated the responses.

When the distance between the d.c. electrodes was short the delays of the late waves obtained in different conditions varied within a narrow range. When the d.c. interelectrodal distance was long some of the late responses, those attributable to the distal pole, occurred with much longer delays than those depending on the position of the proximal pole.

Fig. 4. Appearance of two delayed responses during the passage of d.c. Set-up as in figure 1 except that the distance between the d.c. electrodes was 1.2 cm. and the cathode, instead of the anode, was proximal to the stimulating electrodes. Voltage: 1.0.

A, before; B, during; and C, after application of d.c. D. One thousand cycles.
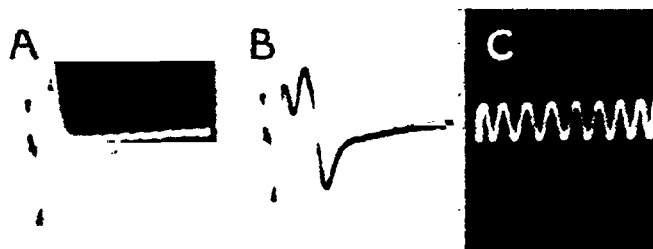
Fig. 5. Late response recorded diphasically between the d.c. and the stimulating electrodes. Excised peroneal from spinal cat. Stimulating electrodes toward the central end and d.c. electrodes toward the peripheral end of the nerve. In A is shown the submaximal early response before the application of d.c. B was taken 2 sec. after an application of d.c.: the early response is followed by a late diphasic excursion with polarity opposite to that of the first response. C. One thousand cycles.

The distance between the stimulating and the d.c. electrodes affected not only the latency, but also the magnitude of the late waves. Thus, if the d.c. and the recording leads were fixed in position, and the voltage and polarity of the d.c. was also maintained constant, shortening the interval between the stimulating cathode and the d.c. electrodes resulted in an increase of amplitude of any late waves present, while lengthening this interval caused a decrease of amplitude. In some experiments, especially if the nerves had been excised for several hours, late responses could only

be seen with relatively short distances between the d.c. and the stimulating electrodes.

If one of the d.c. electrodes was placed at the crushed end of the nerve and the other 1.5 to 3 cm. away on an intact region, only one late spike could be obtained. If the pole in contact with the intact nerve was the cathode the late spike could be readily elicited during the passage of the currents; strong currents were necessary for the additional response with the reversed polarity of the d.c. The late spike appearing at the opening of the current could likewise be seen with either polarity; it was present with lower voltages, however, if the anode, instead of the cathode, was on normal nerve.

In another series of experiments the recording electrodes were both on intact points of the nerve, so that a diphasic record ensued. The advantage of this mode of recording was that it showed, by the polarity of the response ("up-down" or "down-up" on the records) the region of the nerve from which the impulses were coming. If the nerve was stimulated toward the middle and the d.c. was applied toward one end, the record being taken from the other end, the polarity of both the early and the late spikes was the same. If, however, the recording electrodes were at the middle region of the nerve, the other two pairs being toward the ends, respectively, the polarity of the late spikes was opposite to that of the early response (fig. 5).

The conduction velocity of the late responses was measured as follows. The d.c. electrodes were placed toward one end of the nerve. The stimulating electrodes were in the close neighborhood. Records were taken during the application of d.c. with a given voltage and polarity, and with one of the recording leads at the opposite crushed end and the other lead first close to and then far from the stimulating electrodes. The difference in delay between the responses recorded in the two positions corresponded to the conduction time for the stretch of nerve lying between the two points to which the movable lead was applied. The conduction velocities of the late waves thus calculated varied between 50 and 80 m. per sec. in different observations. Specifically in a typical case the conduction velocity of the fibers involved in a given late response, over 4 cm. of nerve, was 63 m. per sec., while that of the fastest fibers contributing to the early response was 88 m. per sec.

In some observations the branches of the peroneal nerve at the lower part of the thigh were separated for about 3 cm. into two bundles. A separate pair of electrodes was applied to each of these bundles in the moist chamber. One of these pairs was used for stimulation, the other for recording. Control tests showed that such stimulation of one of the bundles, even when 4 to 6 times supramaximal, did not cause any responses (by spread) in the other bundle. When d.c. was applied to the peroneal trunk

at a more central region the just maximal or even submaximal stimuli to
one of the bundles caused appreciable responses of the fibers in the other
bundle. Such responses could appear either during or after the application
of d.c. to the main trunk. Their latency depended on the position of the
d.c. electrodes. Figure 6 illustrates an example of this type of experiment.

The experiments carried out on tibialis anticus were as follows. The
muscle was hooked up for mechanical recording. A pair of chlorided
silver electrodes was applied to the superficial branch of the peroneal nerve,
at the ankle. Another similar pair of electrodes was applied to the peroneal
trunk in the thigh; the nerve was cut 3 to 4 cm. more centrally.

The results of a typical experiment are illustrated in figure 7. Short
(about 0.1 sec.) tetanic series of stimuli, with an intensity just threshold
for A fibers, were delivered regularly (about 1 per sec.) to the superficial
peroneal throughout the record. Those stimuli were ineffective except
when d.c. (0.5 v.) was applied to the peroneal trunk, as shown by the lower
signals.

DISCUSSION. Four explanations are suggested *a priori* for the late
responses in figures 1 to 5: *1*, repetitive discharges of some of the fibers
yielding the early spike; *2*, the activation by the stimuli of additional,
slower nerve fibers during or after the passage of d.c.; *3*, the appearance
of axon reflexes in some branched fibers, if present; *4*, the activation of
additional fibers by the nerve impulses set up by the stimuli when those
impulses reach the regions of the nerve where the applications of d.c. have
increased the local excitability.

The explanation of the late waves by repetitive discharges is rendered
quite unlikely by the following considerations. The late waves may be
much larger than the first response (figs. 1 and 4). Beyond a certain
intensity of submaximal stimulation stronger stimuli, which activate more
fibers, evoke decreased late waves (p. 120). Maximal stimulation of the
A fibers fails to elicit any of the delayed spikes. If these spikes were due
to repetition they would increase with the stronger stimuli. Finally, the
experiments made on the split peroneal nerve (fig. 6) and on tibialis anticus
(fig. 7) cannot be accounted for on the basis of repetition.

The explanation that the additional spikes might be due to the stimula-
tion of additional slower fibers is also inacceptable. Maximal test stimuli
would then evoke maximal late effects, but they do not. The delay of the
late responses would depend primarily on the relative distance between the
recording and the stimulating electrodes, whereas this delay was found to
depend upon the relative position of the three pairs of electrodes (p. 121).
The conduction velocity of the fibers contributing to the delayed spikes was
found only slightly less than that of the fibers involved in the early response
(p. 123). The present explanation fails also to account for the observations
made on the split peroneal and on the tibialis anticus.

The third explanation, that the delayed spikes could be due to branches of some axons being activated only in the special conditions caused by d.c. is likewise improbable. In all the experiments, turning the nerve around—i.e., applying any of the electrodes to the central instead of the peripheral end of the nerves—made no difference in the results. It is therefore clear that there was no polarity in the nerves with regard to the phenomenon in question. Were branched axons significant such polarity would be expected. Again, the latency of the responses to axon reflexes would not be mainly determined by the position of the d.c. electrodes, as was found the case.

All the data are in satisfactory agreement with the explanation that the results are due to stimulation of inactive nerve fibers upon the arrival of
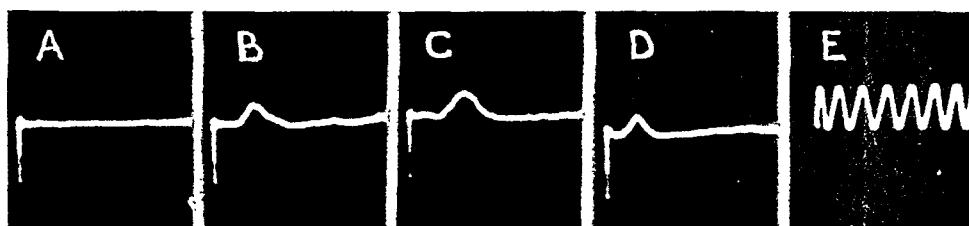
Fig. 6. Activation of adjacent nerve fibers during the passage of d.c. Peroneal nerve with the peripheral branches separated into two bundles. Test stimulating electrodes on one of these bundles and monophasic recording electrodes on the other.

A. Absence of response in the recording fibers upon supramaximal stimulation of the other bundle.

B. Similar stimulation during application of d.c. (1 v.) to the whole peroneal trunk. The cathode of the d.c. was 4 cm. and the anode 5 cm. away from the recording electrodes.

C. As in B, but polarity of the d.c. reversed. The cathode of the d.c. is now distal to the recording leads and the latency of the response is longer.

D. As in B but d.c. electrodes moved 2 cm. closer to the recording leads. The latency of the response is briefer.

E. Sweep calibration, 1,000 cycles.

nerve impulses at a region of the nerve rendered hyperexcitable by the d.c. As Pflüger (1859) showed, the excitability of nerves can be increased at the cathode during the passage of d.c., and at both the cathode and the anode after the current stops flowing. It has been shown recently (Rosenblueth, 1941) that there may also be increased excitability at the anode during the passage of d.c. The appearance of 1 or 2 late waves during or after applications of d.c. is therefore readily explained.

In the experiments illustrated in figure 5 the opposite polarity of the early and late responses is consistent with this interpretation. The first spike traveled from the stimulating electrodes at one end, whereas the late spikes were conducted away from the d.c. electrodes at the opposite end of the nerve.

The changes in latency of the delayed responses with changes in the relative position of the electrodes were precisely as the hypothesis demands. Thus, the maximal intervals between the early and the late responses were obtained with first the recording and then the stimulating electrodes near one end of the nerve, and the d.c. electrodes as far as possible toward the other end.   In these conditions the direct response would be quite prompt, whereas the delayed one would involve the maximal conduction distance, first toward the d.c. electrodes and then away from them, plus the time lost in the activation of the new fibers.   Conversely, if either the recording
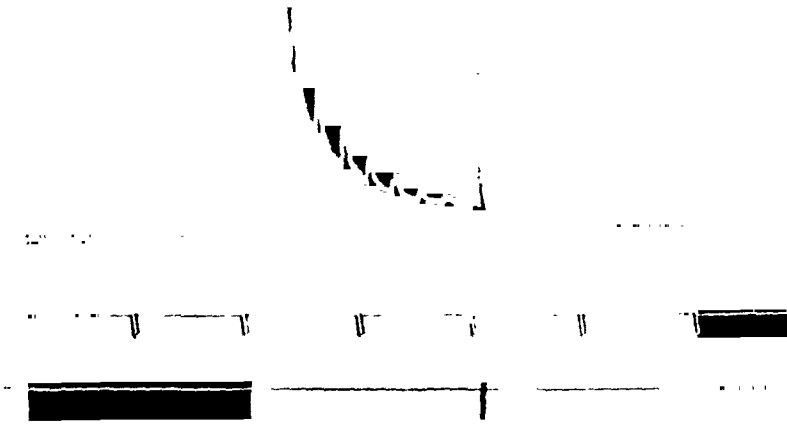
Fig. 7. Decapitate cat.   Record of the contraction of tibialis anticus.   Brief tetani were delivered regularly to the superficial branch of the peroneal nerve at the ankle during the record.   The lower signals show the period of application of d.c. (0.5 v.) to the peroneal trunk in the thigh.   The large responses to the on and the off of the d.c. were cut for reproduction, in order to emphasize the small responses to the test tetani during the period of application of d.c.   Time signal: 5-sec. intervals.

or the stimulating electrodes were close to the d.c. poles, the delay of the late responses was minimal, as is readily understood.

As a final argument in support of the explanation adopted it may be pointed out that it accounts readily for the observations made on the tibialis anticus (fig. 7).   The impulses traveling centripetally over the superficial branch of the peroneal nerve activate the hyperexcitable region of the peroneal trunk treated by d.c.; motor impulses are then conducted centrifugally to the tibialis and a pseudo-reflex is established.   A similar explanation applies to the observations with the split peroneal nerve (fig. 6).

The conduction velocities measured in the excised nerves for both the immediate and the delayed responses (p. 123) indicate that the impulses are carried over myelinated fibers. This is obviously also the case for the experiments on the tibialis anticus. It may therefore be concluded that the myelin sheath does not provide sufficient insulation to prevent the influence of nerve impulses carried by some of the fibers on the adjacent axons in a nerve trunk.

As mentioned earlier, Blair and Erlanger (1932) obtained only negative results when attempting to demonstrate an action of nerve impulses in myelinated fibers on neighboring axons in a trunk. In some of their experiments d.c. was employed, as in the present study, to increase the excitability of the nerve at a given region. Judged from the present data, it is probable that their negative results were at least partly due to the use of weaker d.c. than necessary for the demonstration of the effect. They used subrheobasic intensities, whereas the observations reported here were all made with voltages well above threshold. It is also possible that in some of Blair and Erlanger's experiments the stimulating electrodes were too far away from the region of the nerve treated by d.c. A long distance is unfavorable to the appearance of the phenomenon in question (p. 122). The significance of this influence of distance will be discussed below.

Jasper and Monnier (1938) found delays of about 20 msec. between the arrival of the nerve impulse in one axon at the junctional region and the initiation of the response of the second fiber. Such a prolonged delay is incompatible with the hypothesis that the stimulating agent delivered by the exciting axon is its spike potential. Arvanitaki's (1940) more accurate measurements, however, show delays of 5 msec. or less. His records show further that the spike potential of the stimulated axon develops at the peak of a "local response" (Hodgkin, 1937) similar to that evoked by an electrical stimulus.

The order of magnitude of the axon-axon delay was calculated in this study by subtracting from the total delay for the arrival of the second response at a given point the time necessary for conduction of the initial and the second impulses over the corresponding nerve paths. Such calculations yielded values of not more than 1 to 2.5 msec. in several experiments. These very brief intervals are quite compatible with the hypothesis that the fibers contributing the late responses are stimulated by the spike potential of the fibers involved in the first conducted wave.

This hypothesis is further supported by the influence of the distance between the d.c. and the stimulating electrodes on the amplitude of the late responses. If the stimulus for the second response were any of the other events attending the passage of a nerve impulse (liberation of K, of acetylcholine or adrenaline, etc.) this marked influence of distance would not be readily explained. The only obvious effect on a nerve volley

of a long conduction distance is a slight temporal dispersion. This temporal dispersion will decrease the amplitude and the rate of development of the spike potential at the site of application of d.c. The important influence of amplitude and rate of growth of an electric pulse on its stimulating effectiveness needs hardly to be mentioned.

In describing their observations on juxtaposed unmyelinated axons Jasper and Monnier (1938) spoke of an "artificial synapse." Similarly, Arvanitaki (1940) mentioned a synaptic relationship involving an afferent and an effector axon. The use of such terms in this context is misleading. Transmission across an axon-axon junction and across a synapse may be proved by future experiments to follow analogous mechanisms. To assume *a priori* this similarity of transmission is unjustified. Observations on axons will throw light on problems of synaptic function if synapses turn out to be analogous to axons. Such observations, however, will not be directly relevant to the knowledge of synapses if synapses happen to be different from axons not only in structure but also in mechanism and function, as is suggested by the evidence now available.

SUMMARY

When the excitability of cat's myelinated axons is sufficiently increased at any region by applications of direct current, nerve impulses carried by some fibers stimulate the adjacent fibers (figs. 1 to 7).

The mechanism of this stimulation and the bearing of the data on the problem of synaptic transmission are discussed.

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THE INFLUENCE OF PREGNANCY AND SEX HORMONES ON GALL BLADDER MOTILITY IN THE GUINEA PIG

J. J. SMITH,[1] M. M. POMARANC AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Of the factors believed to play a rôle in gall stone formation, the stasis of bile has always been mentioned as of prime importance. In addition, it has long been noted that gall stones occur predominantly in women of middle age, especially in those who have borne children. A possible relationship between pregnancy and stasis of bile was thus suggested. This subject was investigated radiologically in the human by Gerdes and Boyden (1) who found that of 13 women in the 2nd and 3rd trimesters of pregnancy, all but one showed a decreased rate of emptying of the gall bladder. The mean discharge in these 13 cases, 40 minutes after the administration of the Boyden meal was only 52 per cent of the total gall bladder volume, compared with the nulligravid mean of 73 per cent. Five of these individuals were retested 6 to 8 weeks post-partum and showed marked recovery in the rate of emptying. There is no known explanation for this phenomenon and attention is naturally directed to lower animals in order to study the mechanisms involved.

Mann and Higgins (2) had previously noted that, in the guinea pig, gopher and, with some exceptions, in the dog, the gall bladder of pregnant animals usually does not empty following a fat meal, while in the non-pregnant animals it does. This placed at our disposal a suitable animal for investigation, namely, the guinea pig. In their report Mann and Higgins did not include specific data; therefore it was necessary to repeat their experiments in an attempt to confirm the statement that the gall bladder of the pregnant guinea pig does not empty as well as that of the non-pregnant pig; and further, if a difference existed, to determine it quantitatively.

EXPERIMENTAL. *Gall Bladder Volume of Non-pregnant and Pregnant Pigs—Unequal body weights.* The nature of some of our experiments necessitated the establishment of the normal size of the gall bladder of non-pregnant and pregnant guinea pigs. Throughout the different phases of the work, control animals were used and yielded the results in table 1 for

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[1] Jessie Horton Koessler Fellow of the Institute of Medicine of Chicago.

normal control gall bladder volumes as determined by displacement in S-R solution.

In the non-pregnant animals body weight was found to be the only significant factor influencing gall bladder volume and the correlation coefficient was found to be + 0.470.

In the pregnant animals total body weight was also found to be the most important factor in the determination of gall bladder volume and the correlation coefficient in this instance was + 0.401. A lesser correlation (+0.368) existed between length of gestation and gall bladder size and none whatever (−0.070) between total body weight and length of gestation.

Comment. A rough correlation is thus seen to exist between total body weight and gall bladder volume in both non-pregnant and pregnant

TABLE 1

	NUMBER OF ANIMALS	MEAN G.B. VOLUME (DISP. + S.E. OF MEAN)	MEAN BODY WEIGHT
		cc.	
Non-pregnant..................	29*	1.33 ±0.182	602
Pregnant......................	19	1.90 ±0.162	844†

* Includes 20 females and 9 males.
† Weight includes the products of conception.

TABLE 2

	NUMBER OF ANIMALS	MEAN BODY WEIGHT	MEAN G.B. VOL.
		grams	*cc.*
Non-pregnant....................	15	748	1.75
Pregnant.......................	15	766*	1.80

* Weights include products of conception.

animals, and for this reason, in all experiments in which gall bladder volume was a factor, only guinea pigs of approximately similar body weights were compared.

Equal body weights. To determine whether, on an equal weight basis, the gall bladder of the pregnant was significantly larger than that of the non-pregnant animal, we have collected and compared the gall bladder volumes of 15 non-pregnant and 15 pregnant animals whose body weights were comparable (table 2).

Comment. The data (table 2) indicate that between non-pregnant and pregnant guinea pigs of equal body weights, little or no difference exists in gall bladder volume, when the products of conception are not subtracted from the maternal body weight. Without the products of conception the

pregnant animals in table 2 would weigh less than the non-pregnant animals.

Gall Bladder Emptying in Non-pregnant and Pregnant Animals with Intact Biliary Tracts. Two types of experiments were performed: A, feeding of olive oil, and B, intravenous injection of cholecystokinin.

A. *Feeding of olive oil.* As mentioned above, Mann and Higgins have reported that the gall bladders of pregnant pigs did not empty in response to the feeding of egg yolks and cream. This type of experiment was repeated, using olive oil which in our preliminary tests seemed to produce somewhat better emptying. After a fast of approximately 24 hours, 10 cc. of fresh olive oil were fed. Four hours later the gall bladder was removed and its volume measured by displacement. The resultant gall

TABLE 3

	NUMBER OF ANIMALS	MEAN BODY WEIGHT	MEAN G.B. VOL. AND S.E. OF THE MEAN
		grams	*cc.*
Non-pregnant			
Unfed......................	29	602	1.33 ±0.182
Fed........................	21	555	0.89 ±0.176
Mean emptying.......................................			0.44 ±0.253*
Pregnant			
Unfed......................	14	745	1.68 ±0.162
Fed........................	20	686	1.28 ±0.189
Mean emptying.......................................			0.40 ±0.249*

* S.E. of difference of the two means.

bladder volumes of both pregnant and non-pregnant animals are shown in table 3 with a corresponding group of controls.

Comment. The interpretation of the data and the differences shown is difficult. If one used only visual inspection, as did Mann and Higgins apparently, one might conclude that there was a difference between the gall bladder of the non-pregnant and pregnant guinea pigs after olive oil. This is because after feeding the average volume of the non-pregnant gall bladder was 0.89 cc. and of the pregnant, 1.28 cc., a difference of 30 per cent. But the gall bladder of the unfed pregnant animals was larger than that of the unfed non-pregnant. Calculating the percent evacuation, the non-pregnant animals emptied 33 per cent ± 17 per cent of the bile, whereas the pregnant emptied 24 per cent ± 15 per cent of the bile. The difference is not significant but the trend is toward slower evacuation in the pregnant animals. If one calculated the volume of bile evacuated, a

significant difference is not obtained, but there is again a slight trend toward less bile being emptied by the gall bladder of the pregnant animals.

B. *Intravenous injection of cholecystokinin. Method.* Under sodium pentobarbital anesthesia, the upper abdomen was opened and the incision flaps retracted until the gall bladder could easily be seen. Then, after a short recovery period, 0.5 mgm. cholecystokinin was injected into the femoral vein. The cholecystokinin used was SI, prepared in this laboratory according to the method described by Greengard and Ivy (3). (A preliminary study had indicated that all "non-pregnant" gall bladders responded to this dose of cholecystokinin.) The onset and degree of contraction were noted and 10 minutes after injection the gall bladder was removed and its volume measured by displacement. Eighteen non-

TABLE 4

	NUMBER OF ANIMALS	MEAN BODY WEIGHT	MEAN G.B. VOL. AND S.E. OF MEAN
		grams	*cc.*
Non-pregnant			
Uninjected...................	29	602	1.33 ±0.182
Injected.....................	18	642	0.69 ±0.084
Mean emptying.....................................			0.64 ±0.200*
Pregnant			
Uninjected...................	15	766	1.80 ±0.214
Injected.....................	16	788	1.49 ±0.191
Mean emptying.....................................			0.31 ±0.287*

* S.E. of difference of the two means.

pregnant, sixteen pregnant and three puerperal animals were used in this experiment.

Results. The gall bladder sizes of both pregnant and non-pregnant pigs 10 minutes after injection of CCK are listed with a corresponding group of control animals in table 4.

In one animal in the second day of puerperium and one in the third day, there was no gall bladder emptying whatever after injection of 0.5 mgm. CCK, while in one animal in the sixth day of puerperium there was a prompt and well sustained contraction of the gall bladder.

The intravenous injection of CCK proved to be an effective and consistent stimulus to gall bladder emptying. Of the 18 non-pregnant animals, 11 showed a prompt, easily-discernible contraction of the gall bladder. In these cases white contractile areas appeared on the fundus within 15 to 60 seconds following injection. These areas became larger, coalesced and the gall bladder evacuated most of its contents within 5 to

7 minutes.   In the majority of the remaining animals of the non-pregnant group, the contractions were somewhat slower in onset and seemed to produce a lesser degree of emptying.   In 2 of the 18 there were no visible contractions and no apparent emptying of the gall bladder.

Of the 16 pregnant animals injected, there were no visible contractions in 8 cases; slight contractions, slow in onset and relatively ineffective in 5 cases, and strong, effective contractions in 3 cases.

Comment.   The mean emptying indicated in table 4 is significant in the case of the non-pregnant animals, but not in the pregnant animals, since the gall bladders did not evacuate well in response to CCK. So, both visual and statistical evidence indicates that there is a difference in the response of the gall bladder of the pregnant and non-pregnant guinea pig to an intravenous injection of CCK.   In the same

TABLE 5

PREGNANT ANIMALS	NUMBER OF ANIMALS	LENGTH OF GESTATION	MEAN WEIGHT	MEAN G.B. VOLUME
		days	*grams*	*cc.*
Uninjected*.....................	11		696	1.84
Injected......................	4	15–41	665	1.08
Mean emptying				0.76
Uninjected.....................	19		844	1.90
Injected......................	12	46-65	830	1.62
Mean emptying				0.28

* As mentioned previously, in the pregnant control animals, a higher correlation existed between total body weight and gall bladder volume than between length of gestation and gall bladder volume, therefore the former was used as the basis of comparison with regard to the normal volume of the pregnant guinea-pig gall bladder.

period of time (10 min.) the mean discharge of the non-pregnant gall bladder was approximately twice that of the pregnant gall bladder.

Length of Gestation and Gall-Bladder Response to CCK.   The question now arises as to the influence of length of gestation on gall bladder response.

Of the 16 pregnant animals injected, there were 4 with gestation periods ranging from 15 to 41 days and in all of these there were visible contractions of the gall bladder following injection of CCK; in 3 of the 4, there was definite evidence of considerable emptying.   There were 12 animals with gestation periods ranging from 46 days to term (approximately 64–68 days in the guinea pig) and of these there was visible evidence of contraction in 4 animals, and evidence of marked emptying in only 2 animals. The actual gall bladder volumes after injection of CCK in these two groups of animals, with corresponding control groups, are shown in table 5.

Comment. While the number of animals in the above groups (table 5) is relatively small, the marked difference in mean emptying of the two groups seems to indicate that the response of the gall bladder to intravenous CCK is greater in early pregnancy than in late pregnancy.

Response of Fetal Gall Bladder. It was noted that whether or not the mother had been previously injected with 0.5 mgm. CCK, all fetal gall bladders were uniformly non-contracted. In an attempt to determine if the fetal gall bladder could be made to contract by maternal injection of CCK, 2 mgm. (or 4 times the dose required to contract the non-pregnant gall bladder) were injected into the femoral vein of 5 pregnant animals and the fetuses delivered 15 to 30 minutes later. In no case was there any evidence of contraction of the fetal gall bladder.

Further injection of CCK at this time into the fetal circulation was also uniformly unsuccessful in producing contraction of the gall bladder of the fetus. However, if the newly delivered animal was kept viable for 3 to 4 hours, intravenous injection of small amounts of CCK then produced prompt contraction of the gall bladder.

Comment. The refractoriness of the gall bladder of newly-delivered fetuses makes it impossible to draw any conclusions from the above experiments with reference to the placental transmission of cholecystokinin. Two possibilities are suggested however, to explain this temporary refractoriness to CCK: first, that circulatory adjustments have to be made by the fetus to promote greater blood supply to the gall bladder and permit its contraction, and second, that a chemical or physico-chemical factor in maternal and new-born fetal blood is responsible for a partial or total inactivation of CCK.

Sex Hormones. Although the results of the feeding experiments are inconclusive, the experiments involving injection of CCK give evidence that in guinea pigs *with intact biliary tracts,* there is a decreased emptying power of the gall bladder during pregnancy and this is most marked in the latter part of the gestation period.[2] Such a difference having been established, attention is naturally directed to the cause of the difference. One of the important possibilities would seem to be a direct depressant action of the sex hormones on the musculature of the biliary tract. That these hormones do affect the smooth muscle of various organs is known in the case of the uterus and uterine tubes, and is strongly suggested in the case of the urinary bladder (4) and small intestine (5). The two important mechanisms in gall bladder evacuation *per se,* are the gall bladder muscle itself and the sphincter mechanism at the choledocho-duodenal junction. In the further investigation of this problem, it seemed desirable to determine, if possible, the effect of pregnancy and its hormones on both of these

[2] In addition there is some evidence to indicate that this effect disappears between the 3rd and 6th day of the puerperium.

mechanisms.   The guinea pig was used throughout these experiments and our ·objects have been essentially two-fold: A, to test the *isolated* gall bladder of non-pregnant and pregnant animals and of animals injected with sex hormones; and B, to test the sphincter mechanism of normal and pregnant animals.

Response of the Isolated Gall Bladder of Pregnant, Non-pregnant and Sex-hormone-injected Guinea Pigs to a Standard Dose of Cholecystokinin. Method. Following decapitation of the animal, the gall bladder was removed, the cystic duct cannulated and the organ suspended in a constant temperature bath at 38° according to the method of Doubilet and Ivy (6) for measuring changes in intravesicular pressure.   These authors noted that the optimal intra-gall bladder pressure for an optimal contraction in response to a submaximal dose of the hormone varied from 4.0 to 5.5 cm. of Sollman-Rademaekers' solution ˙placed in the isolated vesicle of the guinea pig.   In our experiments, therefore, the intravesical tension was regulated at this level before CCK was introduced.   The same preparation of CCK was used which has been previously described; 0.25 mgm. of this preparation, added to the 50 cc. bath caused almost all gall bladders to contract submaximally and this dose was used throughout these experiments.   At least 3 tests were made on each gall bladder.   The response was recorded in centimeters of S-R solution.

Five different groups of animals were tested in this manner: I. Non-pregnants—25 animals, 20 females and 5 males. II. Pregnants—26 animals.   III. Castrates—10 animals.   IV. Estradiol injected—10 animals.   V.[3] Progesterone injected—10 animals.

Experiments on castrate animals were performed 7 to 10 days following operation.   The animals of group IV were injected with 7 daily doses of 0.7 mgm. of estradiol, while in group V the total dosage varied from 17.5 mgm. to 35 mgm. progesterone divided into 7 daily doses.   Seven of the 10 animals in group V received a total of 35 mgm.   The hormone injections were begun approximately 7 days after castration, and the animal was used for the experiment 24 hours after the last injection.

Results.   In table 6 are shown the mean elevations of intravesical pressure in centimeters of S-R solution as determined by 3 trials on each gall bladder.

Analysis of the mean elevations of pressure reveals no striking difference between the various groups and only in the case of groups I and IV is the difference statistically significant; the critical ratio in the latter instance is 2.32.

On the possibility that a difference between the response of the gall bladders of pregnant and non-pregnant animals, if it existed, would be more manifest on the first introduction of cholecystokinin, all of the first trials

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[3] Progesterone and Estradiol were supplied through the courtesy of the Schering Corporation and the Ciba Pharmaceutical Company respectively.

were analyzed. It was found that while the response was consistently less in the 1st than in the 2nd and 3rd trials, only a small difference existed between the pregnants and non-pregnants in this respect, the difference being relatively less than that shown in the averages of all 3 trials, as listed in table 6.

In the case of 11 non-pregnant and 12 pregnant animals in the above experiment, pressure readings were taken at 30 second intervals after the introduction of CCK in order to ascertain the rate as well as the height

TABLE 6

	NUMBER OF ANIMALS	MEAN RISE OF INTRAVESICAL PRESSURE (CM. S-R SOL.) + S.E. OF MEAN
I. Non-pregnants......................	25	2.41 ±0.215
II. Pregnants*............................	26	1.89 ±0.286
III. Castrates...............................	10	1.77 ±0.328
IV. Estradiol injected......................	10	1.73 ±0.201
V. Progesterone injected..................	10	1.98 ±0.404

* All except 2 were between 31st and 65th day of gestation.
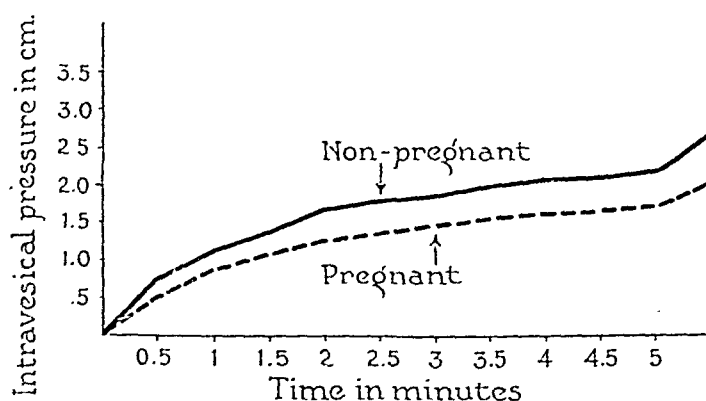
Fig. 1. These curves show graphically the average rate and height of the response of the gall bladder of non-pregnant and pregnant guinea pigs to a standard dose of CCK. The undesignated time shows the maximum response which occurred at some time after 5 minutes.

of response in these two groups. The averaged results are depicted graphically in figure 1.

Comment. A comparison of the response of the isolated gall bladder of pregnant and non-pregnant animals in table 6 fails to give conclusive evidence of a depressant action of pregnancy on the gall bladder muscle itself. The difference between these two groups (I and II in table 6) is not statistically significant (C.R. = 1.818), but the constant slight trend toward a decreased reactivity of the gall bladder muscle of the

pregnant animals makes it difficult to rule out this factor as a cause of decreased emptying power.   Furthermore, an interpretation of the results must take into consideration the natural limitations of the *in vitro* experiment, one of which is the possibility of "washing out" of an inhibitory substance entirely or in part.

In the case of the castrate and hormone-injected guinea pigs, there is also a tendency toward diminished response of the isolated gall bladder.   It is noteworthy that this tendency is greatest and the difference statistically significant, only in the case of the estradiol injected animals, in which the dosage of hormone was relatively large.   This is an interesting result since estrone is generally conceded to increase the irritability of uterine smooth muscle.

It is our opinion that although pregnancy and the injection of progesterone and estradiol caused no marked decrease in reactivity of the *isolated* gall bladder, the observed trend indicates that such a decreased reactivity may be present at least to some degree *in vivo* and may be a factor in the decreased emptying power of the pregnant guinea pig gall bladder.   This interpretation may be supported by the diminished evacuatory response of the gall bladder of the pregnant animal *in vivo* to cholecystokinin.   This is not conclusive however because a diminished evacuatory response could also be due to increased sphincteric tone.

Intra-Sphincteric Pressure of Non-pregnant and Pregnant Animals. *Method.*   Under sodium pentobarbital anesthesia, the abdomen was opened and the common bile duct ligated close to its union with the cystic duct. The common duct was then cannulated toward the sphincter of Oddi and the abdomen closed.   After a recovery period of 1 hour, the cannula was slowly filled with S-R solution until the fluid level fell, which usually occurred suddenly, indicating escape of fluid into the duodenum.   This fall usually continued for several minutes after which time the fluid column stabilized.   Two readings were taken, the first, at the initial fall of the fluid column which represented the pressure at which the solution entered the duodenum, and the second reading, after the flow had stopped and the fluid column had stabilized itself, representing the mechanical pressure maintained by the sphincteric-duodenal mechanism, and which had to be overcome before further emptying into the duodenum occurred.   Three tests were made in each animal, the first reading being taken only on the first test as it was only then that definite values could be obtained.

Results.   The averaged results are shown in table 7.

Comment.   There are only small differences between the two mean pressure readings in the pregnant and in the non-pregnant animals, the differences not being significant in either case.   It is the pregnant animals which yielded the slightly greater values, and it is a matter of conjecture whether or not this represents an actual tendency toward a heightened

sphincteric pressure in the intact pregnant animal. In any event, as determined by this type of experiment, the mechanical pressure maintained by the sphincter mechanism at the choledocho-duodenal junction is not significantly altered in the pregnant animal from the non-pregnant, and seems to give *prima facie* evidence that increased sphincteric resistance is not a significant factor in the decreased emptying of the pregnant guinea pig gall bladder. These animals however were anesthetised.

DISCUSSION. The influence of pregnancy *per se* on gall bladder volume is a question of some interest. Boyden (1) has stated that the pregnant gall bladder is larger than the non-pregnant, basing his conclusion on a study of 5 patients on whom cholecystograms were made during and after pregnancy. In our study of the guinea pig a rough correlation was found to exist in both pregnant and non-pregnant groups between body weight and gall bladder volume. A comparison of the animals of each group having similar body weights showed practically no difference in gall bladder size. Weights of pregnant animals included the products of conception in all cases. Whether the greater size of the pregnant gall bladder is due

TABLE 7

	NUMBER OF ANIMALS	PRESSURE (CM. OF S-R SOL)	
		1st reading (Mean)	2nd reading (Mean)
		cm.	*cm.*
Non-pregnant.....................	12	9.83	5.14
Pregnant........................	12	10.25	5.99

primarily to a non-specific increase in body weight is difficult to state definitely, but our work with the guinea pig seems to indicate that such is the case. This question needs further investigation, preferably using the method of testing the same individuals before, during, and after pregnancy.

Impaired or delayed emptying of the pregnant gall bladder in the human (1), guinea pig and striped gopher (2), has been reported and quantitative evidence submitted in the case of the human (1). That delayed evacuation occurs also in the guinea pig is borne out by our results on the intravenous injection of cholecystokinin. In the dog the effect of pregnancy on gall bladder evacuation is uncertain in our opinion. In the cat according to Whitaker and Emerson (8), pregnancy itself exerts little or no influence on gall bladder emptying. In the latter animal however, Dubois and Hunt (9) have shown that even among non-pregnant members of the species, there is considerable individual difference in the response of the gall bladder to a meal of egg yolk and cream.

Although there are anatomical and physiological differences in the biliary

system of different species, there are, theoretically, three general ways in which uncomplicated pregnancy could bring about an alteration of gall bladder motility.   These may be listed, as follows, along with the possible underlying mechanisms: I. *Interference with* (a) *Production,* or (b) *Transport of cholecystokinin,* e.g., *1,* by an alteration of gastric emptying, or *2,* by chemical inactivation of the hormone in transport.   II. *Inhibition of gall bladder muscle.*   III. *Increased resistance of sphincteric-duodenal mechanism.*

Effects II and III may be produced by: (a) *mechanical factors:* enlarged uterus, and altered intra-abdominal pressure; (b) *changes in amount, physical or chemical character of bile;* (c) *nerve induced disturbances:* Autonomic nervous system and reflexes originating from uterus and pelvic organs, and the G-I tract, especially the colon; (d) *Humoral substances:* Sex hormones and non-specific chemical substances.

Although the effect of pregnancy on fundamental physiological processes has, in a few instances, for example, those dealing with sex hormones and composition of bile, been studied at some length, very little is known of any direct effect of these altered processes on gall bladder activity. That evidence which is available has been summarized in two recent articles (1, 7) and will not be recounted here.

Our experiments with the isolated gall bladder of pregnant and sex-hormone-injected animals demonstrate only a trend toward an actual decreased reactivity of the gall bladder muscle itself.   Likewise the measurement of sphincteric resistance indicates only a slightly increased resistance of the sphincteric-duodenal mechanism in pregnant animals. While it is probable that these factors, singly or together, may be at least partially responsible for impaired emptying of the gall bladder in the intact guinea pig, we believe that these results suggest the existence of another factor, namely a partial inactivation of cholecystokinin in transport.   It is to be emphasized, however, that no direct evidence is available at present to substantiate or repudiate this view.   It emerges as a possibility for investigation due to a lack of any marked inhibition of the isolated gall bladder or of marked increase of sphincteric resistance in the pregnant guinea pig, and it would seem to be compatible with the temporary refractoriness of the fetal gall bladder to CCK immediately following delivery.

SUMMARY

1. Previous studies have indicated that in the human, guinea pig, striped gopher and dog, the gall bladder of pregnant animals does not empty as well as that of non-pregnant animals in response to a meal of egg yolk and cream.

2. Using the guinea pig as the experimental animal, *in vitro* and *in vivo* experiments were carried out in order: *a,* to test the statement that the

pregnant guinea pig gall bladder does not empty as well as the non-pregnant, and *b*, to study the mechanisms involved.

3. In the guinea pig, feeding experiments seem unreliable as a means of detecting differences in degree of emptying of the pregnant and non-pregnant gall bladder, though the results tend to indicate that the viscus of the pregnant animal emptied more slowly.

4. Intravenous injection of 0.5 mgm. cholecystokinin produced prompt and efficient emptying of the non-pregnant guinea-pig gall bladder, as tested in 18 animals, but a delayed and much less effective emptying of the gall bladder of 16 pregnant animals. Some evidence was obtained which indicates that between the 3rd and 6th day of the puerperium, the gall bladder response to CCK returns to normal.

5. In guinea pigs of similar body weights, there was little or no difference in the mean gall bladder volumes of 15 pregnant and 15 non-pregnant animals, when the weight of the products of conception is included.

6. In a series of *in vitro* experiments, the response of the isolated gall bladder of 25 non-pregnant, 26 pregnant, 10 castrate, 10 estradiol-injected, and 10 progesterone-injected animals was tested to a standard dose of CCK. The response was measured in centimeters of intra-gall bladder pressure. In the pregnant, castrate and both hormone-injected groups, the mean values indicated a somewhat diminished response of the gall bladder compared to the non-pregnant control groups. This diminution of response was most marked and statistically significant only in the case of the estradiol-injected animals.

7. The mean resistance of the choledocho-duodenal mechanism was found to be only slightly greater in 12 pregnant animals than in 12 non-pregnant. The difference was not statistically significant.

8. The small differences in the direction of decreased reactivity of the gall bladder muscle and increased resistance of the sphincteric mechanism are, apparently, in part responsible for the impaired emptying of the gall bladder of the pregnant guinea pig.

9. The gall bladder of the guinea pig fetus near term is refractory to cholecystokinin injected into the mother or into the fetal circulation. Three to four hours after delivery the gall bladder responds.

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THE RÔLE OF THE ADRENAL CORTEX AND THE ANTERIOR PITUITARY IN DIABETES INSIPIDUS[1]

MALVINA SCHWEIZER, ROBERT GAUNT, NAOMI ZINKEN AND WARREN O. NELSON

From the Department of Biology, Washington Square College, New York University, and Department of Anatomy, Wayne University Medical School, Detroit

Since the clinical observations of von Hann (1) and the experiments of Richter (2), it has been well confirmed that the presence of a functional anterior lobe as well as the inactivation of the posterior lobe of the pituitary is essential for the maintenance of a permanent, maximal diabetes insipidus. The nature of this anterior-lobe activity has yet to be precisely defined. A specific anterior-lobe diuretic factor has not been isolated, and it is probable that the action is an indirect one. Thyroid maintenance may be involved to some extent through its action on metabolism. The maintenance of the appetite in general by the anterior lobe and the level of the salt intake in particular have been considered as contributing factors. The literature concerning this problem has been well considered in the reviews of Fisher, Ingram and Ranson (3) and Gersh (4).

Silvette and Britton (5, 6), on the basis of indirect evidence, concluded that the factor responsible for the influence of the anterior lobe on diabetes insipidus was support of adrenal cortical function. At the time their work appeared we were investigating this same possibility; and also studying the question of the supposed refractoriness of the hypophysectomized rat to the expected diuretic effects of anterior-lobe replacement therapy. The results of these and related experiments are presented here. An abstract of the earlier experiments has been published (7).

METHODS. In the chronic experiments male rats weighing 150 to 200 grams were used. Continuous daily records were made of body weight, food intake, water intake and urine output through observation periods which were usually of several weeks' duration. In most cases such determinations were also made a week before hypophysectomy for control purposes. Experimental conditions were similar to those previously described (8). Ten per cent sucrose, 1 per cent dry yeast and 5 grams fresh lettuce daily were added to our standard diet (9). Acute tests for antidiuretic sub-

stances were made by the Burn technique as modified by Heller and Urban (10).   Crude anterior-lobe extracts (APE) were made by grinding fresh chilled or frozen glands in sand and centrifuging the debris from a saline suspension.   This material, kept solidly frozen at −5°C., was found to be active for several weeks.
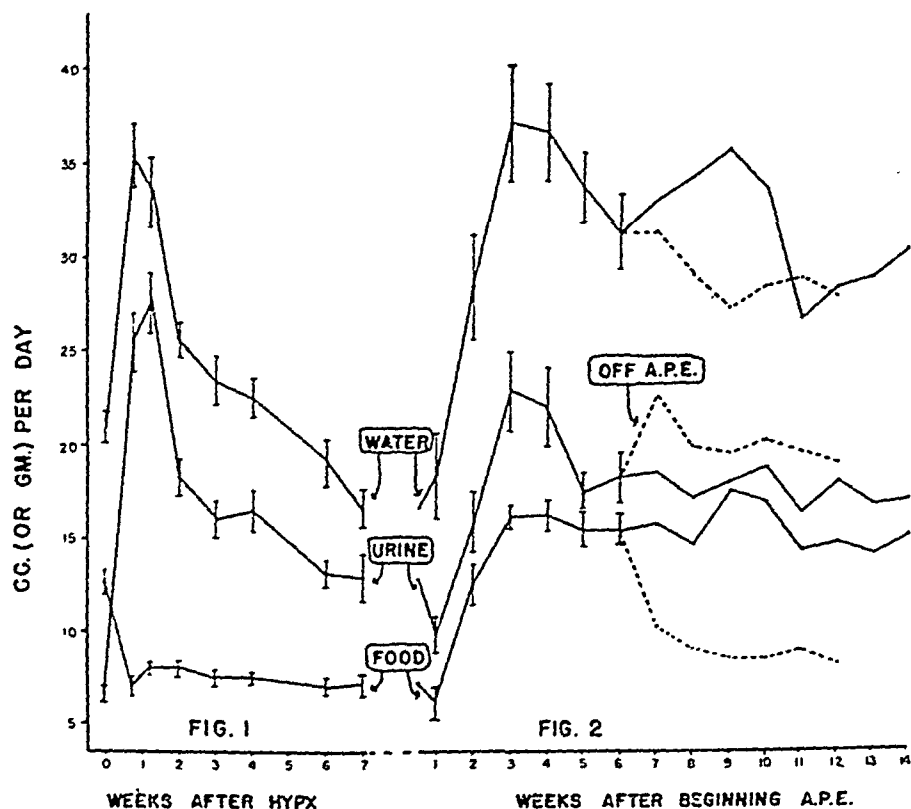
Fig. 1. Average figures on the water intake, urine volume and food consumption of 33 hypophysectomized rats.   Each point is the average for the week indicated with two points included for the first week.   The vertical lines represent standard errors.   Because of deaths and the use of animals in other experiments the number of rats used to determine each point decreases to 22 in the 3rd week and 15 at 6 weeks. If, however, the graphs were made only for the 15 animals followed continuously for 6 weeks no point would vary by more than 2 units from those figured.

Fig. 2. Average effects of crude APE on 8 rats which had been hypophysectomized for 7 or more weeks when the experiment started.   At 6 weeks injections were discontinued in 4 animals whose average records are then shown in broken lines.

The completeness of anterior lobe removal was checked by gross observations at autopsy, by adrenal and gonad atrophy, by a chronic loss of weight, etc.

Effects of hypophysectomy.   In general the nature of the alterations in water exchange following hypophysectomy in the rat reported by others have been confirmed.   Some facts summarized in figures 1 and 3 necessi-

tate further comment.   Because of the inevitably high error of urine volume measurements on small animals, results are frequently reported on the basis of water intake alone.   This practice may give misleading results. On the basis of water intake, our figures would confirm the frequent statement that diabetes insipidus after total hypophysectomy in the rat is only transitory, since thirst eventually returned to normal levels or below.
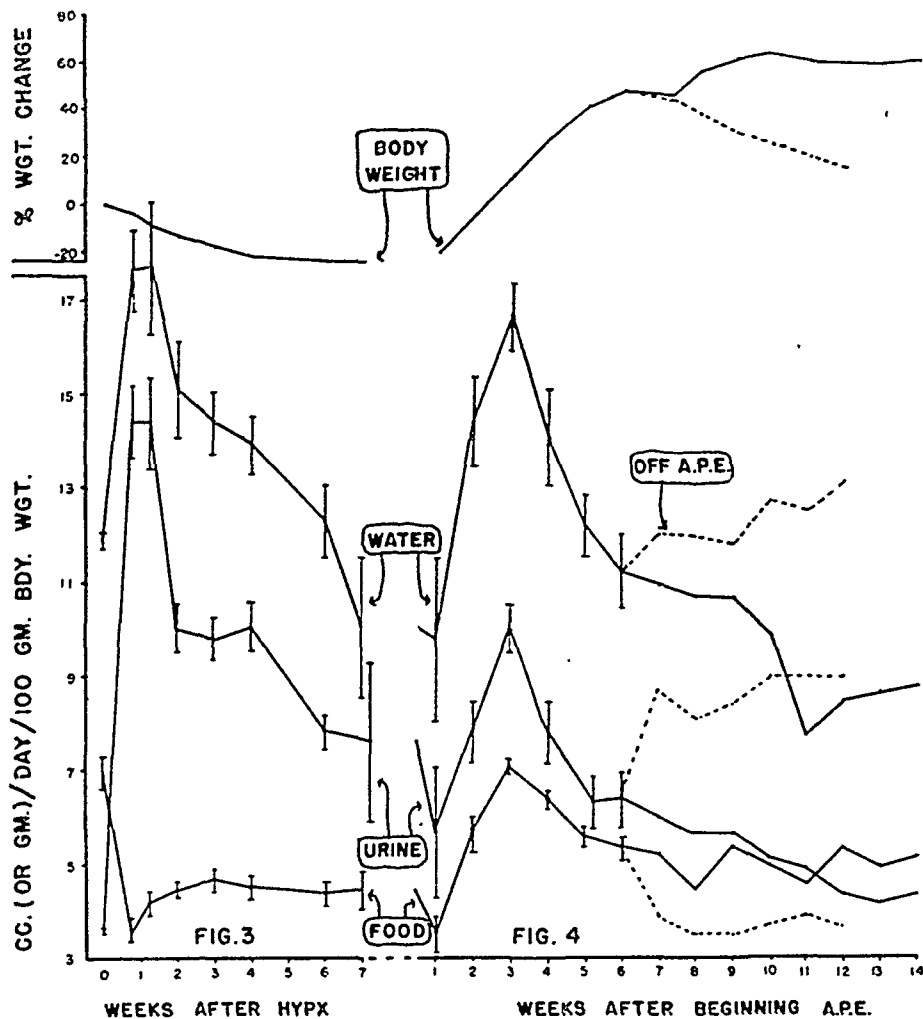
Fig. 3. Same as figure 1, except that results are calculated in grams per 100 grams' body weight.   A curve of body weight change is also included.

Fig. 4. Same as figure 2, calculated as explained for figure 3.

With but rare exceptions, however, urine volumes remained at approximately twice normal levels; and a return to normal was generally associated with moribundity, even in animals on which observations were made for much longer periods than shown in figures 1 and 3.   The diabetes insipidus induced by hypophysectomy in the rat is therefore quickly reduced in intensity but in an attenuated state the polyuria at least is apparently permanent.   Such results are also apparent in the figures of

Dodds et al. (11). The fact that the food intake of these animals was about half normal, and the metabolism no doubt greatly reduced—although it was not measured—enhances the significance of this continued polyuria. Such results also suggest that the hypophysectomy technique used here, for functional purposes at least, effectively eliminated the neurohypophysis.

Phillips and Gilder (12) have also emphasized the permanence of diabetes insipidus in the hypophysectomized rat. They considered, however, that this finding is frequently obscured unless the water exchange is expressed in terms of body weight. This was not true in our hypophysectomized untreated animals; but in treated animals, described below, where excessive growth was induced, absolute figures differ considerably from those expressed in terms of body weight.

Others have found that a high initial polydipsia is not invariable after hypophysectomy in the rat. Richter thinks (2) that this depends upon variable remaining remnants of the neurohypophysis or upon surgical mishaps. Marked polydipsia occurred in about 80 per cent of our cases, but even in its absence a mild polyuria was generally present.

Effects of crude pituitary extract. It has been shown in several species that a near-maximal diabetes insipidus can be maintained after hypophysectomy when APE is given (3, 4). In the rat, however, negative results have attended similar experiments (13, 14, 15) except in one animal (case 107J) of Richter and Echert's series (15).

Eight rats which had been hypophysectomized from 46 to 100 days and in which water exchange had become stabilized at typical levels were injected with crude APE—equivalent of 0.16 gram fresh anterior-lobe tissue daily—for from 6 to 14 weeks. (In addition to the effects reported here the extract would also induce in small dosage a marked enhancement of the glycosuria and ketonuria of partially depancreatized ferrets.) With these doses there was not a complete repair of the atrophic reproductive tracts and adrenal cortices although detailed studies of these effects are lacking as yet.

After an interval of 3 or 4 days during which a reduction of water exchange occurred, a marked stimulation of growth, appetite and water exchange followed in all cases. Water intake reached maximal diabetes-insipidus levels within 3 weeks and then declined. The decline was gradual when expressed in absolute terms, but because of the rapid weight gains was abrupt when expressed in terms of body weight. Urine volumes increased but not to maximal diabetes-insipidus levels. This disproportionate increase in water intake was probably due to a water retention associated with rapid growth. Burn tests indicated some antidiuretic substance in the extract but not in amounts sufficient, had it been pituitrin, to affect daily water exchange in the dosage used here.

By the 5th to 6th week of treatment the secondary decline of water exchange was such that, expressed as a percentage of body weight, it was back to near pre-treatment levels and later dropped still further. The decline was less marked when expressed in absolute terms. This drop in fluid exchange may have been due to an anti-hormone type of refractoriness, but if so it developed only for those factors which maintained water exchange, and not for those which concerned growth and appetite, both of which remained at abnormally high levels.

After six weeks, injections were discontinued in half of the animals and continued in the others. Cessation of treatment evoked a rapid loss of weight and appetite, an increase in urine volume and a slight drop in water intake which was probably insignificant. The augmentation of urine volume was perhaps due to the rapid loss of tissue water coincident with weight depletion. The maintenance of water intake (or its rise in terms of body weight) occurred simultaneously with a sharp reduction in food consumption. This also indicates that the food intake (or the salt content thereof) is not, as some have suggested, a factor of decisive importance in determining the water exchange. These experiments, however, were not designed to furnish critical evidence on this point.

Effects of adrenotropic hormone.[2] Preliminary experiments on 10 animals indicated that the decline in the high diabetes insipidus which follows hypophysectomy could not be prevented by giving adrenotropic hormone (in combination with lactogenic hormone) immediately after operation, in amounts which maintained adrenal weights at above-normal levels (7).

We next used animals that had been hypophysectomized for some time, and in which the initial high water exchange had subsided to see if a diabetes insipidus could be restored. The periods immediately before and after treatment in each case furnished control figures, and were of 4 or more days' duration. A purified adrenotropic preparation was given in doses of either 4, 5 or 10 mgm. per day—amounts adequate to repair adrenal weights to normal after about 7 days' treatment. Since the batches of adrenotropin had different potencies, the effective dosages were approximately identical.

In every instance the effect was clearly to decrease rather than increase the water exchange. The typical ratio of urine volume to water intake seen in untreated hypophysectomized rats remained throughout.

The nature of the antidiuretic action of the adrenotropin is not clear. We found that it was also antidiuretic in the Burn test in intact animals and considered, therefore, that it probably was contaminated with pituitrin. Assays for pressor and oxytocic activity carried out in the research laboratories of Parke, Davis & Co., however, indicated that only negligible

amounts of these substances were present—less than 0.1 pressor and 0.025 oxytocic unit in our daily adrenotropin doses. Injections of pitressin and pitocin in these daily amounts gave slight, if any, effect on the daily water exchange of hypophysectomized animals (table 1). Two units of pitressin per day, an amount 20 or more times that in the adrenotropin, were necessary to depress water exchange as the adrenotropin had done (table 1). Furthermore, the method of preparing the adrenotropic extract was such as to destroy largely its pressor and oxytocic activity. It is possible, if the existence of a questionable separate posterior-lobe anti-diuretic hormone is granted, that such a contaminant may have been the active substance.

According to the principles described by Kárady et al. (16) the chronic antidiuretic activity of this material would not have been due to a non-specific stimulus to antidiuresis seen in the "alarm reaction." Further-more, the mere presence of a foreign substance does not account for the antidiuresis in the Burn technique as our APE was antidiuretic by this test but a similar muscle extract was not.

Whatever may have been the source of this antidiuresis after adreno- · tropin injections, the experiments described below make it doubtful that the action was mediated through the adrenal cortex. Neufeld et al. (17) have also recently observed an antidiuretic anterior lobe product.

Effect of adrenal cortical extract. In experiments similar to the above, cortical extract was injected into hypophysectomized rats to observe its influence on the water exchange. Salt-free extracts, made in the lab-oratory of Dr. W. W. Swingle, were used. Injections were in divided doses—3 cc. per day. This dose was about 6 times that needed for normal growth and appetite in adrenalectomized rats of similar size and age. It was a dose adequate for full protection of adrenalectomized animals during the acute stress of intoxicating doses of water. Larger doses were avoided because of the possible complications of adrenal cortical over-dosage phenomena. As seen from table 1, there was no significant effect on the water exchange. The variations were within normal range.

Similarly in two cases desoxycorticosterone acetate had no effect in doses of 1 mgm. per day (table 1). The decline in water exchange observed in these cases was not unusual for the three weeks for which data are tabulated.

We believe that the doses of cortical extract used here are more than adequate for replacement of cortical function in rats under optimal living conditions. If so, it is clear that the action of the anterior lobe in main-taining a maximal diabetes insipidus is not effected through the adrenal cortex.

DISCUSSION. In all mammals which have been investigated some influence of the anterior lobe is essential for the maintenance of a high

TABLE 1

Effects of various substances on the water exchange of hypophysectomized rats

CASE NUMBER	NUMBER OF DAYS INJECTED	BEFORE TREATMENT		DURING TREATMENT		AFTER TREATMENT	
		Water	Urine	Water	Urine	Water	Urine
Adrenotropic hormone							
1	9	29	22	14	11		
2	6	33	26	20	15	26	24
3	7	35	32	16	13	23	22
4	9	29	22	19	15		
5	4	16	10	10	7	16	13
6	8	26	17	15	10	18	13
7	4	23	17	14	10	25	20
Average............		27.3	20.9	15.4	11.6	21.6	18.4
Pitressin (0.1 U/day) and pitocin (0.025 U/day)							
8	3	17	13	21	12	22	13
9	3	22	13	19	10	18	13
10	6	18	13	14	11	17	13
11	3	21	14	10	7	13	6
12	6	20	12	15	8	15	8
13	3	28	21	31	22	19	13
14	3	19	13	14	10	18	10
15	6	18	10	11	9	17	11
16	6	20	16	14	12	17	13
Average............		20.3	13.9	16.6	11.1	17.3	11.1
Pitressin (2 U/day)							
17	3	31	22	21	16	27	23
18	3	31	22	16	12	29	22
19	3	33	29	22	15	31	21
Average............		31.7	24.3	19.7	14.3	29.0	23.0
Cortical extract (3 cc./day)							
20	7	27	24	22	21	26	23
21	6	18	19	17	19	21	20
22	7	32	27	23	21	21	17
23	5	24	17	14	12	16	10
24	5	30	20	25	20	26	17
25	5	22	16	21	20	25	20
26	5	27	20	32	30	32	26
Average............		25.7	20.4	22.0	20.4	23.9	19.0
Desoxycorticosterone acetate (1 mgm./day)							
27	7	21	15	20	16	20	16
28	7	20	13	17	12	15	11
Average............		20.5	14.0	18.5	14.0	17.5	13.5

diabetes insipidus after ablation or inactivation of the posterior lobe. The results presented here show that the rat is no exception to the rule that anterior lobe replacement effects a heightened water exchange after hypophysectomy. This action of APE in rats is, at least in its maximal aspect, only transitory. The effects of the adrenal cortex on electrolyte and water metabolism invite the hypothesis that the anterior lobe's action in supporting diabetes insipidus is in part its well-known one of maintaining cortical function. Such a possibility was favorably considered on theoretical grounds in the monograph of Fisher, Ingram and Ranson (3), and definitely stated as a theory by Silvette and Britton (5, 6). The data presented here negate this theory. The action of adrenotropic hormone was, if anything, to depress water exchange of hypophysectomized rats; adrenal cortical preparations had no effect; but crude APE reëstablished a maximal diabetes insipidus. Furthermore, other work has shown that under certain conditions the cortical hormone is antidiuretic rather than diuretic (8). Other aspects of the possible interrelationship between the posterior pituitary and the adrenal cortex have been studied by Winter, Ingram and Gross (18).

SUMMARY

1. A polyuria, but not necessarily a polydipsia, is maintained for seven weeks or longer in hypophysectomized rats and is terminated only by moribundity. Water intake alone is not an accurate measure of the altered water exchange of hypophysectomized rats.

2. Contrary to other reports, anterior-pituitary extract will consistently restore diabetes insipidus in long-term hypophysectomized rats to its maximal post-operative levels. Such results are in harmony with those from other species. This effect was not clearly related to growth and appetite responses.

3. The influence of the anterior lobe in maintaining a maximal diabetes insipidus is not mediated through the adrenal cortex. Adrenotropic preparations were antidiuretic in long-term hypophysectomized rats; they did not prevent the subsidence of the high initial diabetes insipidus which follows hypophysectomy; and salt-free adrenal cortical extract or desoxycorticosterone acetate had no effect.

4. The antidiuretic action of adrenotropic preparations, exhibited also in the Burn assay in intact animals, could not be accounted for by their titer of posterior-lobe pressor or oxytocic substances.

ADDENDUM

While this paper was in press two significant articles appeared reporting the production of a polydipsia-polyuria syndrome, accompanied by disturbances in electrolyte metabolism, in normal and hypophysectomized rats (19) and intact dogs (20) receiving large doses of desoxycorticosterone. Using comparable doses we have subsequently obtained similar results on water exchange of hypophysectomized

rats. Such results are not clearly applicable to the problem under discussion here because the doses used (4–8 mg. per day in rats) were almost certainly outside the range of the cortical hormones secreted by the animal with diabetes insipidus even with the anterior lobe intact (see text, p. 146). Ragan *et al.*, in fact, distinguished their syndrome from that of diabetes insipidus.

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THE PASSAGE OF THIOCYANATE AND GLUCOSE FROM THE BLOOD STREAM INTO THE JOINT SPACES[1,2]

J. WALLACE ZELLER,[3] E. G. L. BYWATERS[4] AND WALTER BAUER

From the Medical Clinic of the Massachusetts General Hospital, the Department of Medicine, Harvard Medical School, and the Massachusetts Department of Public Health, Boston

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Previously reported investigations of synovial membrane permeability have concerned mainly the passage of various substances from the joint spaces into the blood stream or lymphatics (1) (2) (3), although passage in the opposite direction is equally important. Quantitative information relative to the transfer of proteins from the vascular system to normal joints and other body cavities has been reported by Bennett and Shaffer (4). The present experiments were undertaken to obtain similar data concerning the passage of two crystalloid substances, CNS ions and glucose.

METHODS. Normal calves were used for all experiments. The animals were bound in a manner which kept them as nearly motionless as possible. That they were not disturbed by the restraining measures was shown by the fact that they frequently slept during the experiments. The synovial fluid aspirations were done aseptically following novocain infiltration of the overlying skin. All synovial fluid specimens were collected from joints proximal to the bindings.

Either 20 to 50 cc. of sterile 5 per cent NaCNS solution or 100 to 155 cc. of 25 per cent glucose solution was injected as rapidly as possible into one external jugular vein. The blood samples were obtained from the opposite jugular vein. Samples of blood and synovial fluid were withdrawn over periods varying from 1 to 26 hours. When periods over 2 hours intervened between the collection of samples, the animals were allowed to walk during the intervals. In such instances water drinking was permitted.

The amount of synovial fluid which could be aspirated usually varied from 0.5 to 3 cc. At times the tarsal joints yielded as much as 6 cc. Although a few of the calves may have become dehydrated during the journey to the abattoir, it was always possible to obtain sufficient fluid for analyses. The amount aspirated at any one time was usually 1 cc., thus leaving some fluid for subsequent removal.

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[3] Nemours Foundation Fellow, 1938–1939.

[4] Rockefeller Fellow (Medical Research Council of Great Britain), 1937–1938.

Most of the specimens were free from blood.   Those which were grossly bloody were discarded; slightly blood-tinged samples were analyzed.

Thiocyanate determinations were made by a slight modification of the method of Lavietes, Bourdillon and Klinghoffer (5), which permitted dilution of the serum and synovial fluid specimens, since at times the latter were less than 1 cc. in volume. We found, as did Lavietes and his co-workers, that duplicate determinations agreed within 3± per cent.

The following chemical methods were used: nonprotein nitrogen, Folin and Wu (6); chloride, Eisenman modification of the Van Slyke method (7); sugar, Folin (8). The protein was determined by a modified macro-Kjeldahl method.   The difference between the total nitrogen and nonprotein nitrogen was multiplied by the factor 6.25 to obtain the value for total proteins.
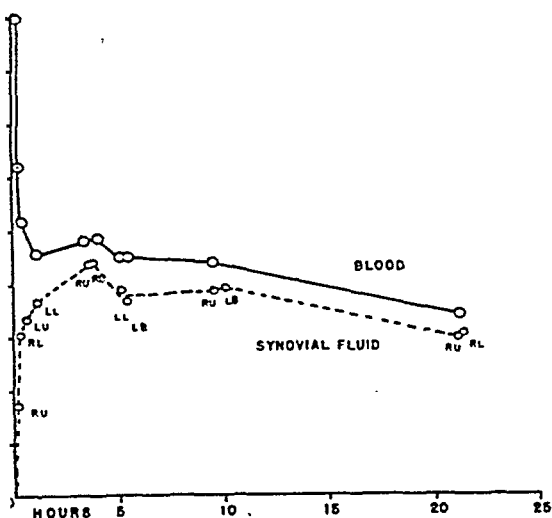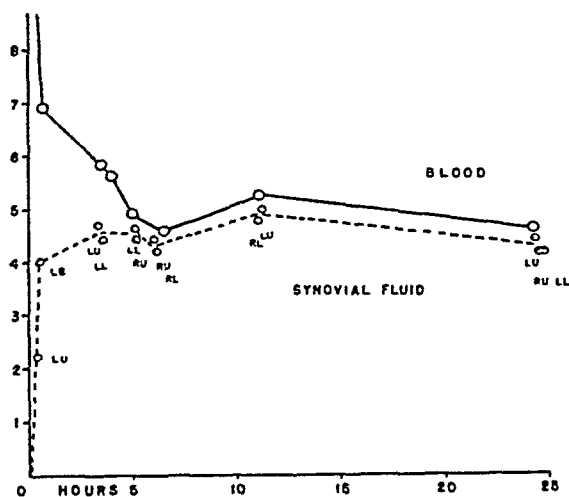
Fig. 1   Fig. 2

Fig. 1. Calf weighed 173 lbs.   Serum and synovial fluid CNS levels following the injection of 28 cc. of 5 per cent NaCNS at 0 hour.   Ordinates, milligram NaCNS per 100 cc.   *LU* and *LL* indicate upper and lower portions of left carpal joints.   *LB* indicates left tarsal joint.   *RU*, *RL* and *RB* indicate similar joints on the right.

Fig. 2. Calf weighed 134 lbs.   Serum and synovial fluid CNS levels following the injection of 20 cc. of 5 per cent NaCNS at 0 hour.   Ordinates, milligram NaCNS per 100 cc.

RESULTS.   *Thiocyanate experiments.*   In 7 experiments the blood and synovial fluid CNS levels were determined at intervals up to 26 hours; in 5 others synovial fluid was removed, as nearly simultaneously as was possible, from a number of joints, in order to determine whether the rate of entry into different joints was the same.

Figures 1 and 2 show that during the first hour following the intravenous injection of NaCNS, there occurred a very rapid rise in the synovial fluid CNS concentration and a correspondingly rapid fall in the blood concentration.   The blood curves then flattened and demonstrated a fairly constant slope for the duration of the experiments.   The synovial fluid CNS rose more slowly and finally assumed a constant relationship to the

blood CNS. The time required for attaining such equilibrium varied considerably. In the experiments illustrated (figs. 1 and 2), it was between 1 and 4 hours and 4 and 5 hours, respectively. In another experiment equilibrium was present in 60 to 70 minutes, and in 4 others it was demonstrable within 2 hours. The longest time required for the attainment of equilibrium was over 6 hours.

The slight increase in the CNS concentration of both the serum and synovial fluid which occurred between the fifth and eleventh hours, as shown in figure 2, is difficult to explain. That it was not due to experimental errors is shown by the unaltered relationship of the serum and synovial fluid CNS levels at the fifth, sixth and eleventh hours. Similar unexplained rises in the blood CNS level have been observed in man and the horse by Crandall and Anderson (9). These workers found that the lymph and gastric secretion of dogs contained more CNS than the blood serum for periods up to 4 hours after injection. It is possible that the secondary rises are due to subsequent rediffusion of the CNS from these body fluids.

The serum CNS concentration remained higher than that of the synovial fluid in all experiments. The difference between the serum and synovial fluid equilibrium levels varied from 0.25 to 0.50 mgm. per cent NaCNS. The average difference was 0.37 mgm. per cent, or 8.8 per cent, in 8 experiments in which the blood was not collected under oil and in which apparent equilibrium was reached.

In one experiment sufficient synovial fluid was obtained to allow simultaneous determinations of CNS, protein and chloride (see table 1). Since the blood specimens were taken under oil, the CNS values expressed in terms of milligrams per 100 cc. of water approximate more exactly those existing *in vivo* than in the other experiments.

The relative concentration of CNS in the cistern fluid, aqueous humor and joints is shown graphically in figure 3. It was lowest in the cistern fluid. This observation is in agreement with that of Crandall and Anderson (9), who found only traces of CNS in the spinal fluid of dogs, 24 hours after intravenous injection. Higher concentrations of CNS were found in the aqueous humor of both eyes. The content of the synovial fluid specimens was even higher and of the magnitude found in previous experiments of like duration. The difference in the serum CNS concentration of the samples obtained in the twentieth and twenty-first hours is far greater than would ordinarily be expected. That the fall in concentration was due to an increased extracellular fluid volume is suggested by the finding of lowered protein and chloride values in the second serum sample (see table 1).

Differences in the rate of entry of CNS into various joints of the same animal could not be consistently demonstrated. In a few instances the

tarsal joints contained less CNS than other joints aspirated simultaneously. The results suggest that equilibrium may be attained more slowly in the tarsal joints, but the number of determinations before the attainment of equilibrium is insufficient to prove this.

In some of the experiments of longer duration, the same joints were of necessity aspirated two or three times. Occasionally, the second or third synovial fluid specimen was cloudy. Smears from such fluids showed numerous polymorphonuclear leukocytes, indicating that the previous aspirations had caused irritation of the synovial tissues. In most cases, these abnormalities were without effect on the CNS levels.

TABLE 1

SPECIMEN	TIME AFTER INJECTION	TOTAL PROTEIN	H₂O	CNS		CHLORIDE		DONNAN RATIO CNS	DONNAN RATIO Cl
				Mgm. per 100 cc.	Mgm. per 100 cc. H₂O	M.eq. per 1000 cc.	M.eq. per 1000 cc. H₂O		
		grams per 100 grams	grams per 100 cc.						
Serum 1.........	19°41′	5.62	94.82	6.50	6.86	99.35	104.7		
Serum 2.........	20°45′	5.30	95.09	5.72	6.02	94.96	99.8		
Joint 1, L.U......	19°46′	1.39	98.42	5.46	5.54	102.9	104.6	1.225	0.998
Joint 2, L.L......	19°49′	1.37	98.44	5.87	5.96	104.0	105.6	1.132	0.986
Joint 3, L.B......	19°51′	1.08	98.68	5.75	5.82	106.7	108.1	1.156	0.962
Joint 4, R.U.....	20°04′	1.38	98.43	5.63	5.72	107.3	108.9	1.146	0.946
Joint 5, R.L......	20°06′	1.43	98.38	5.19	5.28	103.5	105.2	1.236	0.978
Joint 6, R.B......	20°10′	1.12	98.65	5.84	5.92	104.8	106.3	1.094	0.965
Cistern fluid.....	20°30′	0.01	99.59	<.05	<.05	121.0	121.0	121.7	0.828
Right eye........	21°15′			1.84					
Left eye........	21°15′			1.77					

Data obtained in one experiment after the intravenous injection of 50 cc. of 5 per cent NaCNS 42 hours before, and 26 cc. of 5 per cent NaCNS 19 hours and 41 minutes before. The blood CNS and Cl values used in the Donnan ratios were interpolated from the initial and final blood values as determined, assuming that the fall of Cl and CNS was constant.

The available fluid (extracellular) values for 4 calves calculated by the method of Crandall and Anderson (9) varied from 15.7 to 24.4 per cent of the body weight, which is lower than the figure obtained in humans (5), and may indicate that some of the animals were dehydrated when the experiments were performed.

Glucose experiments. Two glucose experiments were performed. In figure 4, the data from the second experiment are presented graphically. The synovial fluid glucose rose more gradually than the synovial fluid CNS. In the first experiment the initial rise of synovial fluid sugar lagged behind that of the blood by at least 20 minutes, differing in this respect

from CNS which could be detected as early as 9 minutes after injection. The blood sugar in both experiments returned almost to the pre-injection levels in less than 2 hours. When the serum glucose fell, the synovial fluid glucose again showed a lag. Similar results in patients with effusions of the knee joints have been observed (10) (11).

DISCUSSION. A constant serum concentration of CNS has been considered by most workers to indicate the attainment of diffusion equilibrium (5) (9) (12). In normal men and dogs and in some of these experiments,
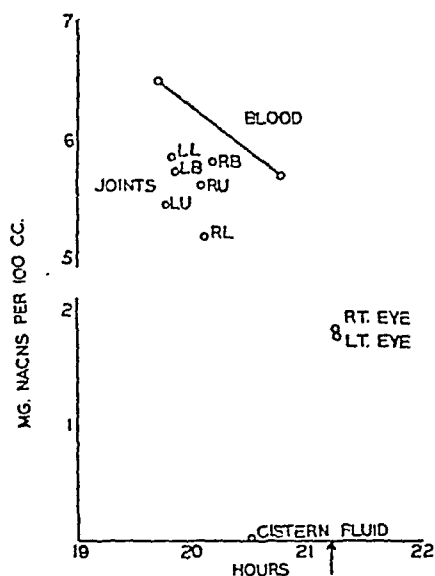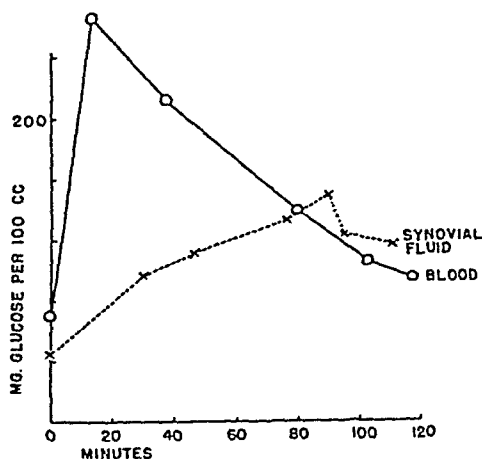
Fig. 3   Fig. 4

Fig. 3. Calf weighed 208 lbs. Thiocyanate concentration in blood serum, synovial fluid, aqueous humor and cistern fluid following the intravenous injection of 50 cc. of 5 per cent NaCNS 42 hours before, and 26 cc. of 5 per cent NaCNS 19 hours before. Abscissae expressed in hours after the second injection. The arrow indicates the time when the animal was sacrificed by section of the large vessels in the neck. Eye fluid was collected immediately afterwards.

Fig. 4. Second glucose experiment. Calf weighed 169 lbs. Injection of 155 cc. of 25 per cent glucose solution at 0 minute. The glucose concentration in serum and synovial fluid for the following two hour period is shown.

equilibrium, as determined this way, is usually reached within one-half to one hour. Despite the initial rapid rate of entry of CNS into the joint spaces of calves, the time at which diffusion equilibrium between serum and synovial fluid is attained is considerably longer than one-half hour and is also quite variable. For example, in one experiment (fig. 1) a relatively constant serum concentration was reached between 43 and 72 minutes, but true equilibrium between serum and synovial fluid was not demonstrable at this time.

The greater volume of fluid (synovia) in the tissue spaces (the joints)

is responsible here for a situation somewhat similar to that found in human patients with edema, in whom equilibrium between plasma and transudates in the subcutaneous tissue, the thoracic and the abdominal cavities usually is not attained until 6 to 10 hours after the intravenous administration of NaCNS (13). Gilligan and Altschule interpret their data from patients as follows: "The time required for attainment of diffusion equilibrium between plasma and edema fluid in a given compartment appears to be directly proportional to the volume of transudate in that compartment" (13).

Their data indicate that at diffusion equilibrium the concentration of CNS in transudates varies directly with the protein concentration and like those of Lavietes, Bourdillon and Klinghoffer (5) show that the average serum CNS concentration is always higher than that of transudates. These differences were thought to indicate that some of the CNS was "bound" to protein or some other relatively non-diffusible substance contained in plasma. Differences between the serum and synovial fluid CNS concentrations of approximately the same degree were observed in the present study.

The Donnan ratios for chloride and CNS are listed in table 1. The ratios for chloride are constant and are similar to those previously found for cattle synovia and other body fluids with the composition of dialysates (14). The CNS ratios vary considerably and average 19.2 per cent higher than those for chloride.

Differences in CNS content of the synovial fluid, the cistern fluid and the aqueous humor can probably be related to differences in protein content and the anatomical and physiological barriers intervening between the blood capillaries and these three body fluids.

Following the intravenous injection of glucose, factors other than simple diffusion of this substance through the body coöperate in rapidly lowering the blood sugar. When this occurs, the rate of utilization of glucose by the articular tissues and the rate of diffusion from the joint space into the synovial capillaries are not sufficiently rapid to keep the synovial fluid sugar level at or below that of the serum. Similar glucose differences between blood and pleural transudates in diabetic patients with cardiac failure have been described (15). Glucose equilibrium was not attained in the present experiments. The fact that glucose enters the joint space more slowly than CNS suggests that glucose equilibrium is reached later than is that of CNS. The validity of this assumption can only be tested by simultaneous experiments of longer duration on the same animal.

It is probable that substances resembling CNS and glucose in molecular size and physical composition, whether necessary for or detrimental to the economy of the intra-articular structures, can enter the joint spaces. Since previous experiments have shown that compounds of small molecular

dimensions are promptly transferred from resting joints to the blood stream (16), it is readily understood why normal synovial fluid is kept in balanced equilibrium with the blood. Further studies of this nature are necessary if a better understanding of articular physiology in health and disease is to be obtained.

<div align="center">CONCLUSIONS</div>

1. Thiocyanate ions and glucose diffuse readily into the joint spaces of calves following intravenous injection, CNS entering more rapidly than glucose.

2. Thiocyanate diffusion equilibrium between serum and synovial fluid is usually attained in from 1 to 4 hours following the intravenous injection of NaCNS.

3. At equilibrium the concentrations of NaCNS in sera not collected under oil averaged 8.8 per cent higher than the synovial fluid concentrations.

4. Thiocyanate diffuses into the joint spaces in larger amounts than into the anterior chambers of the eyes. Only traces enter the cistern fluid.

5. These studies indicate that some of the CNS is held in the blood in a non-diffusible state.

6. With respect to CNS and glucose the equilibrium between serum and synovial fluid resembles the equilibrium between serum and transudates in patients with edema.

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THE RELATION OF THE Q-T INTERVAL TO THE REFRACTORY PERIOD, THE DIASTOLIC INTERVAL, THE DURATION OF CONTRACTION AND THE RATE OF BEATING IN HEART MUSCLE

H. A. BLAIR, A. M. WEDD AND A. C. YOUNG

From the Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The present work was undertaken as a preliminary to the study of the action of drugs on the heart using the action potential (Q-T interval) as an index. It was considered desirable for this purpose to know the relation between the absolutely refractory period and electrical changes, the relation between electrical changes and the duration of contraction of the muscle, and the variation of electrical effects due to heart rate. We have studied these relations in strips of turtle heart and have made a few observations of the recovery of the Q-T interval in man after exercise. For convenience we have used the term Q-T interval for the interval between depolarization and repolarization of the heart strips in analogy to the common electrocardiographic nomenclature.

Various measurements indicate that the absolutely refractory periods of tissues are related to the intervals between the depolarization of the cell membranes consequent to excitation and the subsequent repolarization associated with recovery. Tait (1910) first seems to have suggested a connection between the refractory phase and the electrical manifestations. While this is agreed upon in general there is considerable disagreement as to the exact relation. Adrian, for example, (1921) concluded that the absolutely refractory phase corresponded closely with the duration of the monophasic action potential in frogs' nerve and heart but not in skeletal muscle. Gasser and Grundfest (1936) recently confirm Adrian's view for nerve. In the heart, however, it was indicated by earlier work of Trendelenburg (1912) and deBoer (1915) that the absolutely refractory period might outlast considerably the monophasic electric response. There are certain difficulties arising in all attempts at correlation. In the case of nerve in particular, it is not safe to assume that the monophasic action potential wave corresponds exactly to the period intervening between depolarization and repolarization at a certain region. In the case of the heart, additional uncertainties are introduced because not only does the

slowness of conduction enable different regions to be excited and to recover at quite different times, but it is well known that all parts of the ventricle do not remain active for the same length of time (Einthoven, 1913). Thus the region at which the stimuli are applied may have a longer or shorter refractory period than the region at which electrical measurements are made. When the electrogram of the whole heart is used this effect is exaggerated because the electrical activity begins with the reaction of the first region to be excited and ends with the recovery of the last region to be repolarized.
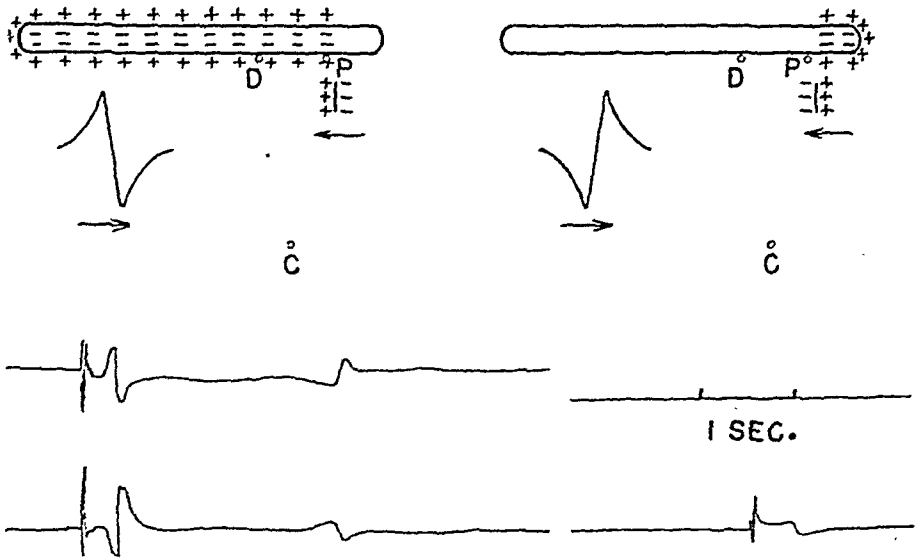
Fig. 1. The upper half of the figure illustrates the theory of the method described in the text. On the lower left is a record of the electrical changes from two neighboring points of a ventricular strip. From left to right the first complex is due to the electrical stimulus, the following interval is the conduction time to the recording electrode. The first diphasic wave is due to depolarization which is maintained for some time. Subsequent repolarization is indicated by the second diphasic wave. On the lower right is a similar record from a spontaneously beating auricular strip. The timing indicated applies to both records. The Q-T interval is measured from the point where the middle stroke of the depolarization wave crosses the base line to the similar point where the repolarization wave crosses the base line.

It appears that the only satisfactory way to solve this problem in heart tissue is to use a method which records the potential changes at one region and measures the refractory period simultaneously as near as possible to that region.

EXPERIMENTAL METHOD. In the turtle heart experiments, strips of the ventricle or auricle, usually about 2 cm. in length and 2 or 3 mm. in width, were used. Except in the experiments in which contraction was studied, the strips were dissected at least 1 day and sometimes 2 or 3 days before using. For the purely electrical experiments they were set up on filter

paper on a paraffin block and kept irrigated with Ringer's solution. The strips were stimulated by means of two electrodes placed transversely at one end. Near these (see fig. 1) usually about 1 mm. distant, was one lead-off electrode P while another D was situated about 1 cm. distally. Both of these electrodes touched the strip while a third C was placed about equi-distant from P and D and about 5 cm. away. A separate amplifier with an ink recorder was connected to measure the potential differences occurring between each of the pairs of electrodes PC and DC. The pair PC recorded electrical variations near the stimulated region and the pair DC recorded the changes in the distal region if these were desired.

For the experiments in which contraction was studied simultaneously with electrical changes the muscle tension was recorded photographically using a glass rod as a lever of the so-called isometric type. In these experiments shorter strips, about 1 cm. long, were used to reduce the time of conduction. These strips were used within one to several hours after dissection. They were immersed in a small bath of Ringer's solution, but the distant electrode C (fig. 1) was still placed on a field of filter paper running out from the edge of the bath. In order to relate records of contraction and electrical changes which were taken simultaneously but recorded separately, the stimulus was recorded on each and each was accurately timed.

Outline of theory. The theory of the potential variations with the electrode arrangement used is illustrated in figure 1. If, as shown on the left side of this figure, a muscle bundle is stimulated between the electrode P and the right hand end, depolarization will occur at this end. The subsequent changes of potential as depolarization proceeds will be entirely equivalent to those produced by the passage along the bundle of the dipole shown underneath (Craib, 1927, 1930; Wilson, Macleod and Barker, 1933). The potential of the distant electrode C will remain approximately at the same value as that of the mid region of the dipole, which may be taken as zero. Thus P will be positive, zero, and negative, respectively, with respect to C as the dipole approaches it, is opposite to it, and recedes from it. A record of the potential of P with respect to C will, therefore, be like the diphasic curve drawn under the bundle. A record using electrodes D and C will have the same form but will occur later.

In a muscle bundle such as we used all of the fibers are depolarized and appear to remain in this condition for some time before repolarization commences. If the bundle recovers from the stimulated end the potential of P with respect to C will undergo, as illustrated on the right hand of figure 1 by an oppositely directed dipole, a series of changes similar to those occurring during depolarization but in the reverse order, P being first negative to C and later positive. If, on the other hand, recovery begins at the end last excited, i.e., on the left in the figure, the recovery will be in the same

order but will reach D first. Thus depolarization and repolarization will give rise to a record from either pair of electrodes PC or DC consisting of two diphasic waves separated by an interval which measures directly how long the depolarized condition lasted at P. It should be emphasized that the equivalence of the dipole to the partly polarized cell is in no wise altered by such external conditions as the amount of fluid surrounding the muscle or its conductivity, etc. These conditions will, however, determine the magnitude of the potential difference recorded between electrodes and also the proper position for the electrodes in the field. In the experiments to be described, it is implied that the muscle is surrounded by a homogeneous field and that the electrode C is essentially normal to the muscle both at P and D and that the front of the wave of depolarization is at right angles to the axis of the muscle bundle. These requirements, for various reasons, will usually be met sufficiently well although seldom exactly. It is implied also in figure 1 that in a given region both the depolarization and repolarization are abrupt rather than gradual processes. Which they are is not known for single cells, but heart strips repolarize more gradually than they depolarize as is evidenced by the fact that the amplitude of the recovery wave is different from that of the depolarization wave. This effect in bundles might be due to the cells being out of step in their abrupt recovery or it might be due to the recovery being progressive at each region of each cell. Because of this, some uncertainty is introduced in deducing the exact timing of the electrical events at a given point. This is not serious in the present work because the interval between depolarization and repolarization is much larger usually than the time required by either process.

EXPERIMENTAL RESULTS. *Type of record.* In figure 1 (lower left) is given a pair of records from a strip of turtle ventricle using electrodes such as PC and DC. It will be seen that there are two diphasic waves as expected but the recovery wave is small in amplitude denoting less abrupt repolarization than depolarization (see Wilson et al., loc. c., p. 37). Figure 1 (lower right) is from a spontaneously beating auricular strip. It may be said that in general the depolarized condition does not last nearly as long in the auricle as in the ventricle. Most of our records are of this general character although there are minor variations in form. In particular, either the depolarization or the repolarization wave may have one of its phases greater in amplitude than the other. There is seldom any doubt, however, about the choice of the point denoting the passing of the middle of the dipole, i.e., the point at which the wave swings across the base line from the one side to the other. It should be emphasized that we present curves of the expected form not to support the dipole theory outlined above but only to indicate that the experiments are adequate. Assuming the validity of the membrane hypothesis the dipole theory is a

direct deduction from electrical potential theory which is based in turn on experiments much more conclusive than living tissue is likely to provide. When the record is not in accord with the dipole theory it simply means that there are divergences from the experimental conditions postulated. The only question that can arise apart from experimental conditions is whether or not the muscle bundle acts sufficiently like a single uniform cell with the same orientation to enable one to use the dipole theory in interpreting the record. In our experience it does act sufficiently like such a cell, provided there are no newly cut or injured regions in the bundle.

Refractory period. To determine the refractory period the shocks were usually about five times the normal threshold strength. These will determine a period somewhat greater than the absolutely refractory period but only slightly so as the early relative refractory period shows very rapid recovery. Stronger shocks were avoided because of their probable effect on the repolarization process. While making these measurements the heart was driven at a constant rate and extra test shocks were placed nearer and nearer to the repolarization wave until no extra response occurred. After the extra shock gave rise to a response it was necessary to allow the Q-T interval to return to a value normal for the steady driving rate before testing again.

In table 1 are given several sets of data from ventricular strips. In some of these cases (in which the recording was done so close to the stimulating electrode that the Q wave and the shock were not separable) the time from the last normal shock to the extra shock is compared with the time from the last normal shock to the recovery wave. In other cases the Q wave was separate and the shock to extra shock interval was compared with Q-T. Both of these methods are essentially the same. Usually the time for the effective shock is taken as a mean of the times to a just-effective and to a just-ineffective shock. Since the extra shock distorts the T wave the normal shock to T interval is taken from the previous normal beat. It will be seen that the absolutely refractory period coincides with the shock to T interval quite closely except in the one set; in this the point of recording was not close to the point of stimulation. We conclude from these results that the tissue at a given region is absolutely refractory while it is depolarized. Different regions of the same strip may be depolarized for different lengths of time. Consequently both types of measurement must be made at the same region as nearly as possible in making a valid comparison. Failure always to respect this requirement may account for the disagreement between various workers.

Q-T interval and diastolic interval. While driving the heart at a regular slow rate the Q-T interval was studied as a function of the diastolic interval for a single interpolated shock. A typical set of data is shown in figure 2

in which the Q-T interval for the beat following a single early shock is plotted against the interval from the preceding T wave to this shock.   This

TABLE 1

EXPERIMENT	SHOCK TO SHOCK	SHOCK TO T	EXPERIMENT	SHOCK TO SHOCK	Q-T
	seconds	seconds		seconds	seconds
1	2.38	2.28	3	2.24	2.16
	2.28	2.28		2.24	2.20
	2.32	2.28		2.07	2.00
	2.15	2.04 D		1.92	2.04
	2.02	2.04 D			
2	2.52	2.50	4	2.39	2.20
	2.08	2.12 D		2.14	2.00
	2.04	2.08 D		1.78	1.68 D
				1.86	1.70 D
			5	1.66	1.70
				1.74	1.80
				1.34	1.48
				1.44	1.68
				1.02	0.80

Several sets of data relating the Q-T interval to the absolutely refractory period. The one column gives the minimum interval from the usual to the test shock.   The other column gives the interval from the usual shock to the T wave of the resulting response when the shock and Q are inseparable, otherwise it gives the Q-T interval. Each group of measurements is on a different tissue.   Each pair was taken at different times.   The results marked D were obtained after the Q-T interval had been shortened by digoxin.   In experiments 1 to 4 the electrical measurements were made within 1 or 2 mm. of the stimulating electrode.   In experiment 5 the electrogram was taken from a region about 1 cm. from the locus of stimulation.
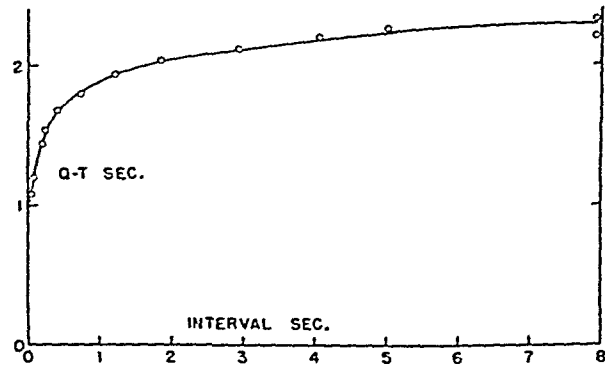
Fig. 2. The Q-T interval for a single early beat plotted against the interval between the last regular T wave and the shock eliciting the early beat.

relates the variation of the Q-T of any early beat with the earliness of the beat.   In this particular experiment the intervals between the T waves

and the succeeding shocks during the steady driving were 8 seconds. A still slower rate would probably not bring about any greater lengthening of Q-T. A single extra shock put in after 5 seconds' interval changed the
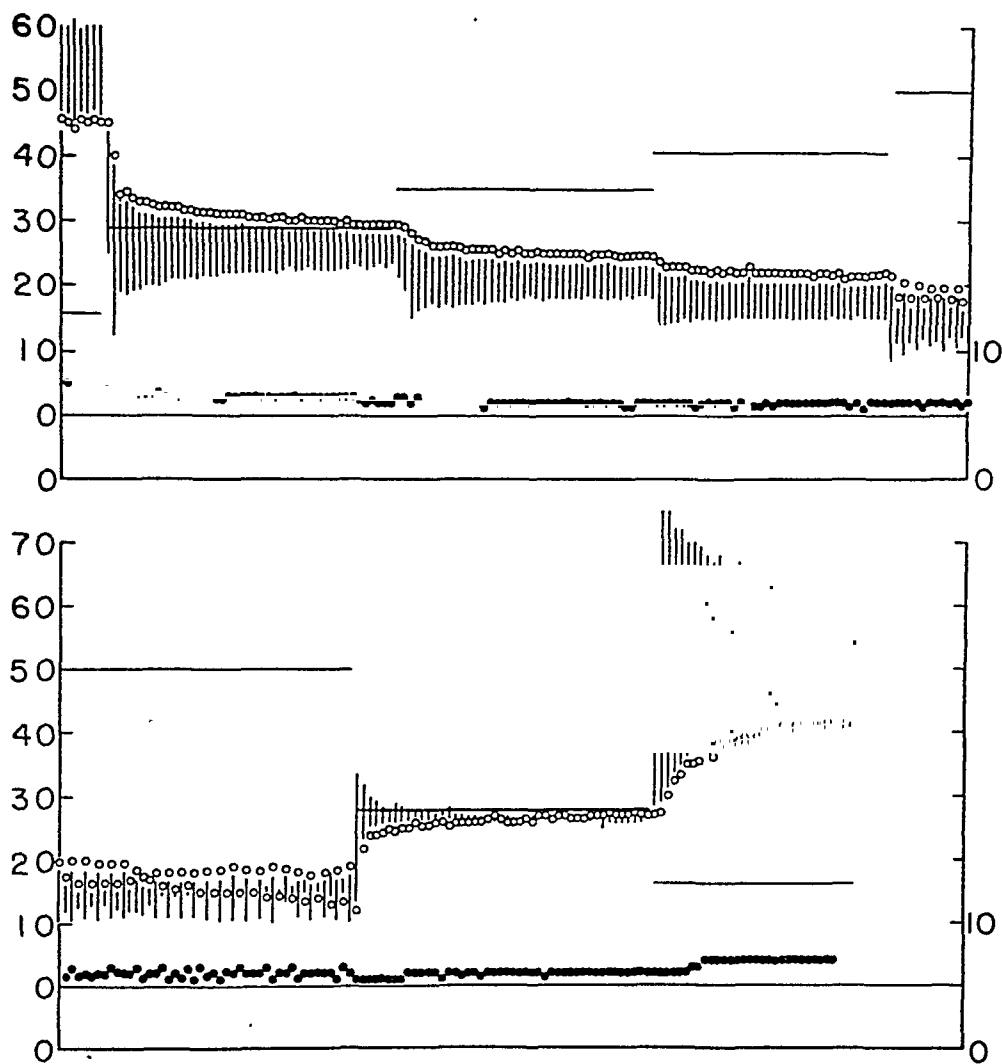
Fig. 3. On the upper ordinate scale are plotted on an arbitrary scale Q-T intervals, circles, and T-Q intervals, the ends of the vertical lines farthest from the circles. With each Q-T interval is plotted the succeeding T-Q interval. The driving shock lies within the T-Q interval. The rates in beats per minute are given, according to the ordinate scale, by the positions of the horizontal lines. The number of beats at any given rate can be found by counting the beats under these lines as each successive beat is plotted for the whole experiment. The dots plotted on the lower ordinate scale are conduction times between the shocks to the Q waves.

ensuing Q-T very little. But as shorter and shorter intervals were tried, marked shortening of Q-T occurred until finally an extra shock placed just after the T wave caused the ensuing Q-T to be only about half as long as the original. Thus the well known dependence of the Q-T interval

on heart rate is due not only to the cumulative effects of rate but to each disatolic interval as it occurs.

Q-T interval and heart rate. Cumulative rate effects do exist, however. In figure 3 are plotted Q-T intervals and T-Q intervals for successive beats during a series of increasing then decreasing rates. Starting at about 16 beats per minute and then changing the rate to 29 shortens Q-T markedly at first, in keeping with the shortening of T-Q, the phenomenon just considered. In addition, however, it will be seen that Q-T now slowly shortens to a new steady level. This process allows T-Q to lengthen, because the two intervals together must equal on the average the constant shock to shock interval which determines, in turn, the rate. The shortening of Q-T would be somewhat faster if T-Q did not lengthen.

On increasing the rate further it will be seen that a similar quick change and then drift of Q-T occurs each time until a rate of 50 is attained. Then the intervals are unable to stabilize. At the beginning of this phase the first T-Q is quite short, consequently the next Q-T is short; because of this the next T-Q is long and this in turn lengthens the next Q-T and so on. Ordinarily this reciprocal action produces only a small movement in or out of the T wave and stabilization is produced analogously to that determined by a mechanical governor. In the present case, however, the T-Q interval is of the order of 0.5 second. This value is at the bend in figure 2 where a small change in T-Q produces a large change in Q-T. In consequence of this there is an overshooting of each interval due to a change in the other and stabilization does not occur. Attempts to drive the heart strip under these conditions usually result sooner or later in a response being missed, and then a two to one rhythm obtains.

On decreasing the rate to 28 per minute it will be seen that Q-T interval lengthens appreciably at once and then gradually drifts toward a still greater value. On further slowing to 16 per minute, the original rate, Q-T no longer responds as promptly to the increased diastolic interval but moves continuously toward a greater length. At the end of the experiment it is still 10 per cent shorter than the original. Complete recovery might eventually occur but not necessarily because of the observed tendency for Q-T to shorten slightly, 5 per cent or 10 per cent per hour even with steady driving at 15 to 20 per minute.

Heart rate and conduction time. The interrelation of heart rate and conduction time was also studied in the foregoing experiment and is illustrated in figure 3. On the lower ordinate scale from 0 to 10 are plotted the relative times from the shock to the Q wave for each of the beats represented above. Each dot represents the conduction time for the beat succeeding the diastolic interval plotted directly above, i.e., for the beat whose Q-T interval is plotted in the next column. It will be observed that the expected shortening of conduction time with increase of rate is

usually obtained.   There is some tendency during the period of irregular beating seen on the lower left of the figure for conduction to be faster after a short diastole and slower after a long diastole.   Thus conduction may also be influenced by the diastolic interval per se as well by the rate of beating.   Its dependency on diastolic interval is not always parallel to that of Q-T, however, because if the shock comes very early after the T wave the conduction then occurring during the relatively refractory period is very slow again.   In addition it will be observed in the last two stages of the experiment that the slowing of conduction is considerably delayed and then is suddenly completed within one and three beats respectively.   Such observations make it difficult to draw definite conclusions about the precise relation of conduction to either the diastolic interval or the rate of beating.   It should be stated that the term conduction time as used above and later includes any electrical latent period there may be as well as true conduction time.   Our observations lead to the conclusion that the latent period, if it exists at all, is a negligible part of the whole time.

Heart rate and Q-T interval in human subjects.   In order to see whether the drifting of Q-T discussed above would occur in the human heart, several subjects were studied.   The heart rate was changed by a short period of exercise.   Figure 4 gives a typical example of the results.   The beat intervals and the Q-T intervals are plotted here for successive beats, first for a short normal period before and then for several minutes after exercise.   It will be seen that following exercise the heart rate is higher and the Q-T interval considerably shorter.   When the heart rate has slowed to normal the Q-T interval is still short, but it continues to lengthen until it attains the value 40, about 10 per cent super normal.   It retains this value at the end of the experiment even though the rate, after having been somewhat slow for a time, is again at the original level.   It is suggested by these results that the Q-T interval may be a more sensitive index of the state of the muscle following exercise than is the heart rate. It is evident that the Q-T interval in the human heart is subject to influences other than those which are directly related to rate.   Consequently there can be no exact general relation between heart rate and Q-T interval alone.   Numerous formulae purporting to express such limited relationship have been submitted.   It is obvious from the present observations, however, that such formulae must contain unexpressed relations concerning the equilibration of Q-T at each rate.   It would be misleading, therefore, to consider them to be other than empirical relations of practical usefulness.

Q-T interval and contraction.   In keeping with the idea that the Q-T interval or refractory period of cardiac tissue outlasts the rising phase of contraction so that tetanus cannot occur, it is stated that, for the heart in situ, the T wave usually coincides with the closure of the aortic valve.

However, attempts to show exact correspondences between electrical and mechanical events have led, on the whole, to the conclusion that they do not exist (Katz, 1928). This is to be expected, perhaps, because any definite relation between contractility and electrical activity can apply to the local
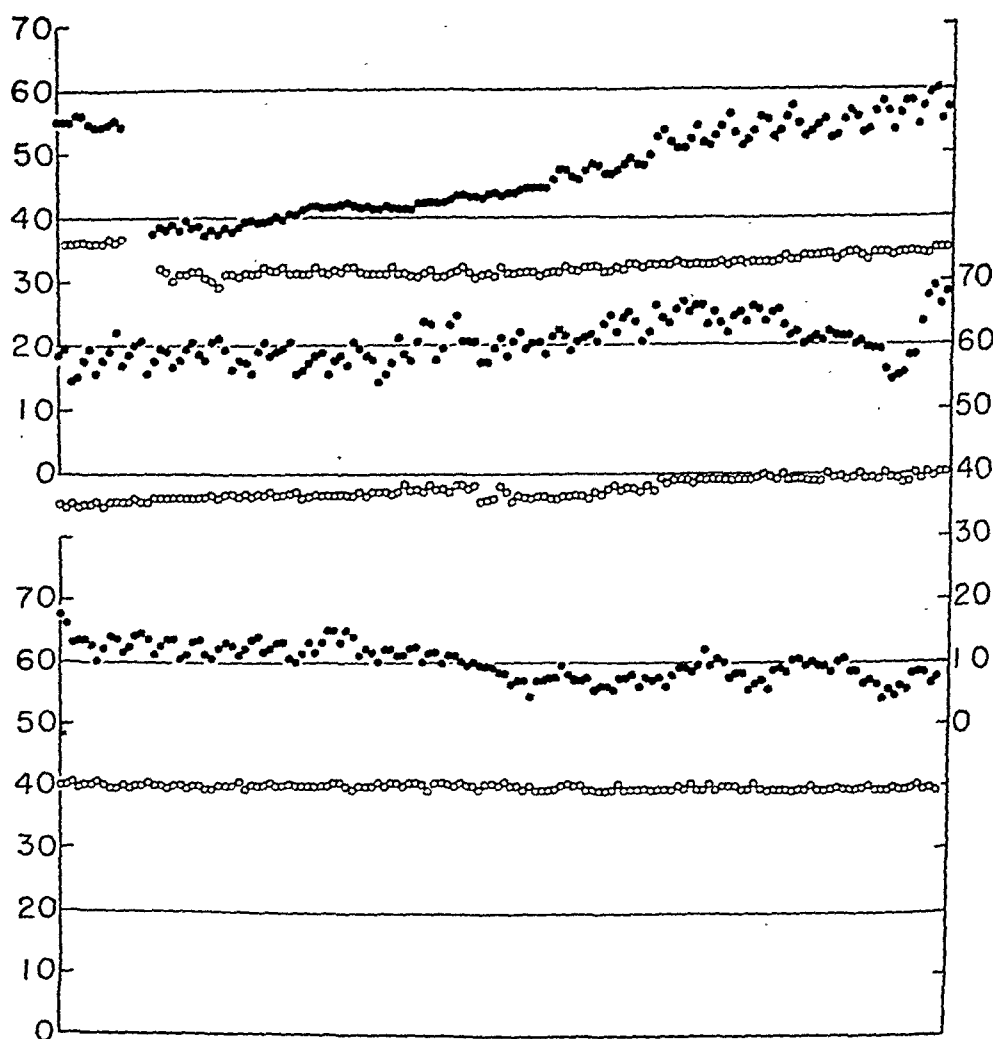
Fig. 4. The intervals between beats, Q-Q intervals, in dots plotted along with the included Q-T intervals in circles for a human subject preceding and following a short period of exercise indicated by the break near the top left. The data of successive beats are plotted before and after the exercise. The ordinate scale for the middle part of the figure is given on the right hand side. The ordinates are in arbitrary units.

region only. In the whole heart both electrical and mechanical events occur in an order determined by a conduction process, and the duration of activity at different regions is different. Consequently any local intrinsic relation is not only likely to be submerged in the activity of the whole

organ, but there is no great likelihood that two different hearts will integrate their activities in exactly the same way.   For these reasons we deemed it advisable for the most part to seek a relation between Q-T and the mechanical events in short strips of tissue in which conduction time is relatively short.   The essentials of the method were described above.

In figure 5 is a set of mechanical records of a ventricular strip driven at four different rates.   It will be observed that the beats are markedly shortened in duration and decreased in height as the rate is increased.
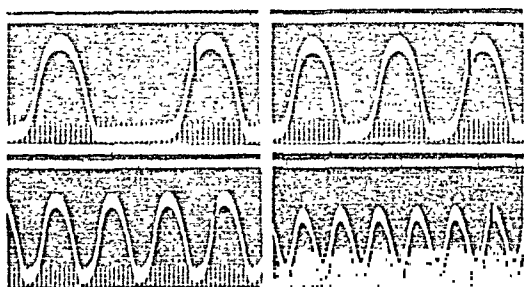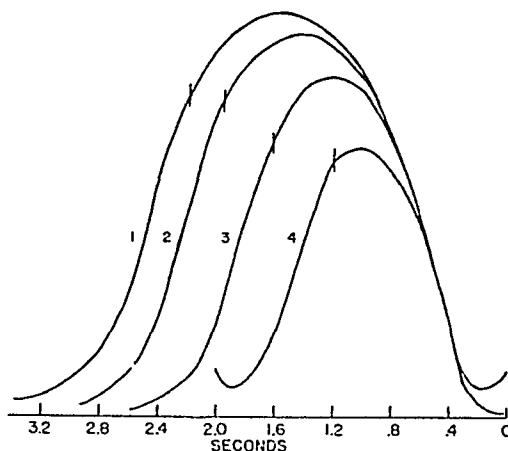
Fig. 5   Fig. 6

Fig. 5. Isometric contractions of a strip of turtle ventricle at four different driving rates.   The rising phase of contraction goes from right to left following the stimulus which is recorded by the break in the horizontal line above.   The stroke on the falling phase of a representative curve of each class marks the time of occurrence of the T wave.   This is taken from a record like figure 1 and is located by the stimulus which is common to both records.   The interval between the vertical timing lines is 0.2 sec.

Fig. 6. The marked curves of figure 5 are plotted on the same scale so the early rising phases coincide.   The two middle curves had to be moved, 0.04 sec. in the case of the larger and 0.06 in the case of the smaller toward the left in order to get coincidence.   For these curves the shock would occur at 0.04 and 0.06 sec. respectively, for the others at zero time.   The vertical strokes mark the times of the T wave. Relaxation was incomplete at the highest rate.

This is shown more clearly in figure 6 in which the curves are drawn so as to coincide during the rising phase.   In order to accomplish this the two curves at the next to the lowest and next to highest rates respectively had to be shifted 0.04 and 0.06 second toward the left.   These curves rose slightly earlier than the slowest, probably because conduction improved with increase in rate.   The fastest curve matched the slowest without shifting.   It should be emphasized that over a wide range of the rising phases one can choose a tension common to all the curves and hold that at this particular tension the rate of contraction is the same in each.   This

similarity in the early stages of contraction leads directly to the conclusion that the contractile process begins unaffected by the change in rate of beating. Consequently the effect of rate is to modify the later stages of contraction only, and one is led to seek relations between the rate of beating and the duration of the contraction.

To study this the position of the T wave is marked on one curve of each set (fig. 6) by laying off on the mechanical record the shock to T interval of the corresponding electrical record. It will be seen that following the T wave relaxation is rapid in all curves and runs a similar course in numbers 1 to 3. Therefore, some event occurring about the time of the T wave reduces the muscle to the same state in all cases except that at the fastest rate. In that instance relative prolongation of conduction, compared to the duration of the beat, probably accounts for the lower relaxation. This relation of the T wave to relaxation suggests that the repolarization indicated by the T wave stops the contraction process. The alternative suggestion that preparation for rapid relaxation elicits repolarization is weakened by the fact already emphasized that the contraction in each case started in the same manner and presumably would have continued the same in the absence of interference. But if repolarization stops the contraction process, it is necessary to account for the fact that slow relaxation has been taking place for some time before the T wave is written. In other words, it must be shown that repolarization commences soon enough before the point where the T wave crosses the line in changing phase, which point is taken for the time of writing of T, to account for the initial decline of tension from the peak of the curve. With the present method of recording it does, and this is illustrated in figure 1. The T wave here begins to rise about 0.5 second before its reversal. The beginning of T must be due, however, to the existence of repolarization somewhere. Therefore, repolarization must have started at least 0.5 second before the middle of the wave. This time can be deduced more exactly as follows: preceding the T wave repolarization must have started at the first point to recover the sum of the time required for it to go to completion at that point and the time required for conduction from that point to the recording electrode. This repolarization time at a given point is the interval between the peaks of the diphasic T wave at that point. In figure 1, for example, this time is approximately 0.15 second while the conduction time for depolarization (shock to Q interval) is approximately 0.33 second. In this case, as can be seen from the two records together, the T wave is conducted at about the same rate as Q, and repolarization begins 0.48 second before T. For the data of figures 5 and 6 the corresponding time is 0.4 second. This estimate is only approximate because it assumes that recovery started at the same place as excitation and traveled at the same rate over the same path. None of these assumptions is necessarily valid,

and in fact it is rather unlikely that the T wave will always travel at the same rate as Q because, as is well known, it moves much more slowly in the intact ventricle.   Therefore the time given, 0.4 second, is the least time that the beginning of repolarization can precede T.

Recording of T at the point of stimulation will not solve the difficulty for it cannot be safely assumed that recovery will necessarily begin at the first point excited. Moreover if it did, repolarization would spread evenly from the region of the contact electrode and a diphasic record would not be obtained.   Furthermore, since the contraction cannot be localized to the point of electrical measurement, conduction effects are unavoidable. For these reasons we stimulated in the middle of 1 cm. strips and recorded electrically from about the middle of each end section.   If, as was usually the case, the T wave was written about the same time from each half, repolarization probably started at the middle of the strip.   Consequently the T wave indicated that about one-half of the strip had become repolarized.

As stated above, repolarization in the experiment of figure 5 started about 0.4 second before the T wave.   Of this time 0.25 was required for conduction to the middle of each end section.   A similar additional time would be required for complete repolarization to go the remainder of the way to the two ends.   In considering the range of influence of the T wave, it may be said to extend backward about 0.4 second or 0.25 second depending on whether the beginning of, or the completion of repolarization, respectively, is the more important for relaxation.   Applying these ideas to the curves of figure 5 the T wave follows the peak of the curve at the slowest rate by 0.6 second.   Consequently in this case if repolarization stopped the contractile process it must have begun to do so at least this early.   With the other curves their contraction can be said to be altered at the point at which they start to diverge from the slowest curve.   Curve 2 ceases to coincide with 1 at 0.86 second before its T wave.   Curve 3 diverges from 1, 0.74 second before its T wave while curve 4 diverges from 1 about 0.66 second before its T wave.   These divergences occur considerably sooner than 0.4 second, the latest predicted beginning of repolarization.   This is possibly due to the slower conduction of the T wave than of that of Q, which was the only one measured.   This type of correlation is difficult to establish particularly in ventricular strips for a number of reasons.   There is a source of error, for example, in this experiment in that a considerable number of beats were recorded at each rate, and in going from the slowest rate to highest successive curves may be lowered somewhat by fatigue.   It will be seen from figure 6 that small decreases in height of the middle curves in particular would cause marked changes in their points of departure from the uppermost curve.   An increase of conduction rate of T as well as Q in going from the second curve to the third

may account for the relatively earlier T wave in the latter.   This will not account for the T wave of the fourth curve being relatively earlier than the third, but in this case the point of departure is not definite.   We have found in other experiments with longer ventricular strips that conduction changes with rate of beating altered the contraction rate too much to make possible the type of comparison of the beats used above.

These possible sources of divergence should be reduced in the experiment recorded in figure 7 in which an auricular strip was used.   In the auricle the conduction rate is much faster and the experiment was done so as to
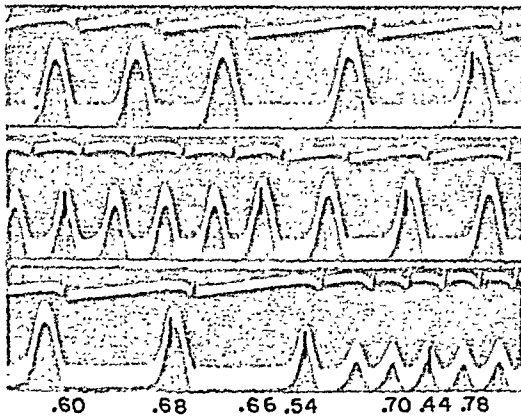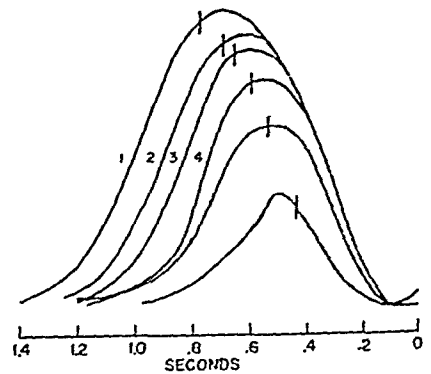
Fig. 7   Fig. 8

Fig. 7.   A series of contractions of an auricular strip driven at different rates.   The records go from right to left.   The stimulating shock is indicated by the beginning of the break in the upper line.   The form of this line is not determined by the shape of the shock but by the capacity of the recording circuit.   The upper two records are consecutive, a few beats are omitted between the second and third.   The vertical lines mark the times of occurrence of the T wave in the several curves whose shock to T intervals are given numerically immediately below.   The rate at the upper right and the lower left is 17.6.   The fastest rate on the lower right is 63.   The longest vertical lines mark 0.2 sec.

Fig. 8.   The marked curves of figure 8 drawn to the same scale and placed so the shock is at zero time for all.   The T wave is indicated by a vertical line in each curve.

cover the series of rates in a much shorter time.   In figure 8 these data are plotted as in the previous experiment.   Now, however, none of the curves are shifted to allow for conduction change.   It will be seen that the four slowest curves 1 to 4 coincide in the initial portions indicating that here again the contraction starts off independent of rate of beating.   The two fastest curves show, however, marked slowing of conduction.   The influence of the T wave on contraction is again indicated by the points of divergence of the second, third and fourth curves from the first.   In this experiment the intervals from these points to the T waves are 0.18 second, 0.19

second and 0.2 second respectively. These increase slightly as the rate increases, contrary to the results with the ventricle. This time 0.2 second corresponds to about the beginning of repolarization in the auricular strip, as deduced by the argument used above for the ventricle. Repolarization time, and conduction time in particular, are shorter here. This experiment which can be given more weight than the previous one on the ventricle, supports the view that the beginning of repolarization inhibits the contraction process.

It should also be noted in figure 7 that although the heart rates at the upper right and the lower left of the figure are the same the contractions are quite different while their shock to T intervals are different. The contraction at the lower left is very similar to that of the middle right hand two curves for which the rate is much faster. These latter sets of curves are similar while their shock to T intervals are similar. These results can be taken as further indication that the contraction is not controlled by the rate directly but through the effect of rate on the shock to T interval.

Another point to be noted in figures 6 and 8 is that the time of appearance of the T wave bears no relation to the extent of contraction. Such evidence is very much against the idea that repolarization is set off by the contraction process having reached a certain stage.

Further evidence that the polarization controls contraction is given by experiments with digoxin. This drug will markedly shorten the shock to T interval. At the same time the duration of the beat shortens even though the rate is maintained constant. These data will be presented at another time.

Another method, rather less direct, of relating Q-T and contraction leads to the same conclusions. The basis of this method is illustrated in figure 9. If the horizontal line here represents the beginning and duration of Q-T at the proximal region of the contracting strip, the upper line will serve the same purpose for a distal region, and intermediate lines could be drawn for intermediate regions. The conduction time of Q will be given by Q-Q and the conduction time of the recovery wave by T-T, made equal to Q-Q for simplicity. Contraction will begin just after the first Q, and its subsequent course will depend in part on the local properties of the muscle and in part on the conduction rate. The rise of tension may be represented by the curve on the lower left of figure 9. According to the hypothesis adduced above, relaxation will be initiated by repolarization, and its subsequent course will be determined by the conduction of the repolarization wave and the various mechanical properties of the muscle. The decline of tension may be represented by curve 1 on the right hand of figure 9. If now Q-T is shortened at all points to a new value Q-T$_2$ leaving conduction the same, it follows from the hypothesis that the new relaxa-

tion curve 2 will be of the same shape as 1 but earlier in occurrence by the
same amount as T_2 is earlier than T_1.   And for any further shortening of
Q-T the same will be true providing conduction is essentially the same.
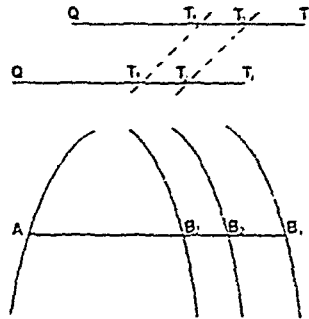
Fig. 9. Description in text
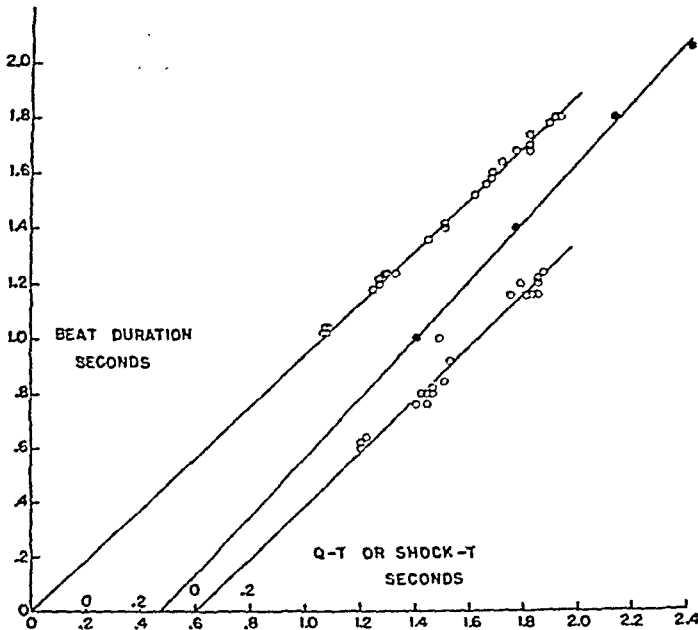
Fig. 10. The left hand and right hand curves are the Q-T intervals as abscissae and
the widths in seconds of the contractions—the quantities AB of figure 9 as ordinates.
These data are for consecutive beats of ventricular strips driven at increasing, then
decreasing rates in experiments analogous to those of figure 7.   The middle line is
for the data of figure 5.   In this case shock to T instead of Q-T is plotted.   The width
of the curves of figure 5 was measured at a level corresponding to about one-half the
height of the lower right hand set of curves.   The origins of abscissae for the three
curves are given by the three 0 points in the same order from left to right.

Consequently any line AB measuring the width of the contraction in
seconds at a given tension level will be shortened in proportion to Q-T.

A convenient way of testing this hypothesis is to plot the width AB
against Q-T to see if a straight line is obtained.   This is done in figure 10.
It will be seen that the two sets of data plotted with Q-T intervals as ab-

scissae fall closely on straight lines going through the origin.   Consequently the duration of contraction as measured here is directly proportional to Q-T and the hypothesis that Q-T controls contraction is substantiated further.   The other curve in which shock to T is plotted is also linear, but, as is to be expected, it cuts the axis of abscissae at a point corresponding to the shock to Q interval.   These data are therefore in agreement with the hypothesis also.   The only widely diverging datum observed is the one point belonging to the curve on the right.   In this case the beat is too wide for the Q-T.   This beat followed a period of rapid beating after a pause of several seconds.   It was considerably enhanced in height even over the normal at very slow rates.   This exception may be due to alterations in conduction.

Conduction changes occurring equally for Q and T probably would not disturb the relation of Q-T to contraction because they would tend to alter the slopes of the rising and falling phases of figure 9 equally, leaving their separations essentially the same.   Such changes occurred in the data of figure 10.   If the conduction of Q and T are altered disproportionately, however, the beats concerned will no longer be comparable.   The exceptional beat in figure 10 probably belongs to this class.

It will be seen from figure 9 that if the duration of the beat measured at the tension at which the line AB is drawn is proportional to Q-T this could not still be true if the duration of the beat were measured lower down. For a duration measured lower down the lines of figure 10 would still be straight, but they would not pass through the origin.   The beat durations for the line going through the origin in figure 10 are approximately equal to the Q-T intervals.   If Q starts contraction and T ends it, there should be some line AB for which A is the average time of beginning of contraction of the various regions of the muscle and B is the average time for relaxation, and this line AB should then be equal to the average value of Q-T.   If all the Q-T intervals are equal any one is an average.   Consequently, according to the hypothesis and as realized in figure 10, a beat duration at the proper tension level is the average contraction time for the muscle elements. Thus a definite meaning in conformity with the hypothesis can be given to the beat duration measured from about the middle of the rapidly rising phase of contraction.

Conclusions regarding Q-T interval and contraction.   From these results we conclude that the contractile process, which is released by the depolarization wave consequent to excitation, proceeds as long as the membrane remains depolarized.   When the membrane begins to repolarize at a given region the contractile process in that region is either retarded or arrested.   If retarded only, it is almost certainly completely arrested by the time repolarization is complete.   Thus polarization governs contraction, not contraction polarization, and the effects of rate are primarily on

polarization.   It cannot be denied, that there is a third factor determined by the heart rate which simultaneously arrests contraction and starts repolarization.   This indeed seems probable, but in the absence of any direct knowledge of the relation between excitatory and contractile processes there is no present ground for speculation on this point.

Relation to skeletal muscle.   The above conclusions are quite different from those which can be drawn from skeletal muscle.   With that tissue repolarization is complete in less than 1 millisecond, i.e., before contraction starts.   It may be that contractile energy is released only while the membrane is depolarized and that its action persists for about 0.05 second after its release, this being the approximate duration of the rising phase of contraction.   In the heart it may be true also that the contraction energy is not all expended as released, but may persist in its action for a similar time after repolarization commences.   This time is too short, however, to have any discernible effect in the heart by present methods of observation.

Q-T and contraction in the intact heart.   The lowest curve of figure 7 illustrates the difficulty which may be encountered when the conduction time is long either on account of low velocity or long distance.   In this case repolarization follows so closely on depolarization that relaxation begins at the first point stimulated very soon after contraction begins.   In consequence no great amount of the muscle is contracting at a given time, and the tension never gets very high.   On the other hand, contraction exists somewhere for a relatively long time because the conduction is so slow.   In the whole ventricle the conduction time for depolarization (Q-R-S interval) is relatively short, but the conduction time for repolarization (duration of the T wave) is long.   Changes in the former will probably have only small effects on contraction under most conditions, but changes in the latter may be quite important.   This possibility makes it unlikely that a definite relation between the T wave and contraction will hold for all heart rates.   The inverted T wave may provide another difficulty. This is an index that the wave of repolarization travels in the same direction as the wave of depolarization instead of contrary to it.   Since repolarization now proceeds from inside to outside of the ventricle relaxation of the muscle presumably proceeds in the same way.   Consequently it may not be assumed without investigation that the inverted T wave will bear the same relation to contraction of the whole organ as does the erect T wave.   On the whole, however, since the peak of the T wave probably indicates the time at which repolarization is proceeding in the bulk of the muscle, it is to be expected that relaxation of the bulk of the muscle will bear a fairly definite relation to it.   The relation of the Q-T interval to the duration of the beat as evidenced in figure 10 contains much more promise for practical use than the position of the T wave alone.   It is very probable that this relation will ordinarily hold for the whole heart.

SUMMARY

In turtle heart strips the electrical activity is recorded from pairs of electrodes one member of each pair being against the tissue and the other at a distance. The record permits the measurement of the interval between the depolarization and the repolarization of the tissue at a given region. This interval is called the Q-T interval. It is shown to coincide with the absolutely refractory period. It is shortened to one-half or less of its maximal value in a single very early beat. Further slow shortening occurs when the strip is driven for periods at a series of increasing rates. The lengthening of Q-T on slowing the rate is a slower process. Evidence is presented that repolarization arrests the contractile process in the muscle leading to the conclusion that the electrical processes control the mechanical rather than the mechanical, the electrical. In this connection it is shown that the duration of contraction as measured, for example, from half contraction to half relaxation is related linearly to the Q-T interval over a wide range. In the human heart it is shown that in recovery from exercise there is no fixed relation between the Q-T interval and the rate.

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THE EFFECT OF CHOLERESIS ON THE RATE OF EXCRETION OF INTRAVENOUSLY INJECTED BILIRUBIN

A. L. BERMAN, E. SNAPP AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

This investigation was undertaken for several reasons. *First*, relatively little is known regarding the rate of excretion in the bile of intravenously injected bilirubin. However, considerable is known regarding its disappearance from the blood plasma of man and animals (1, 2) because bilirubin clearance is used as a liver function test (3, 4). The rate of removal of such a substance from the blood plasma does not mean that it is eliminated in the bile at the same rate. *Second*, the effect of choleresis produced by the intravenous injection of a bile salt on the rate of elimination of intravenously injected bilirubin in the bile has not been studied. *Third*, the effect of choleresis, similarly produced, on the rate of removal of bilirubin from the blood has not been adequately studied. Dragstedt and Mills (1, 5) found in a few experiments that the intravenous injection of sodium dehydrocholate had no effect or appeared to slightly augment the rate of removal of intravenously injected bilirubin, whereas in patients the injection of sodium dehydrocholate (Decholin-Sodium) has been reported to elevate (6) and to decrease serum bilirubin (7). *Fourth*, whether the administration of bile salts to patients after the release of a common duct obstruction or with retention of bile pigment due to other causes will increase the rate of excretion of the retained pigment is an open question. Sodium dehydrocholate, however, is used clinically for such a purpose, even though it is known that, under normal conditions in the dog, the production of a choleresis by bile salts does not increase the total daily output of bile pigments in the bile (8, 9). *Fifth*, since bile contains other pigments than bilirubin and *since the pure bilirubin prepared from bile for intravenous injection has different solubility properties from "bilirubin" in bile*, we have studied the rate of removal of pigment with and without choleresis when bile was injected intravenously.

METHODS. Adult dogs weighing between 12 and 15 kilos were used. The animals were anesthetized with nembutal (sodium pentobarbital), using 64 mgm. of the drug per 5 lbs. The abdomen was opened, the common duct was cannulated for the collection of bile, and the cystic

duct was ligated.   In 19 experiments *the kidney pedicles were ligated* tightly with linen thread so as to eliminate any possible loss through the urine (12). Pure indirect-reacting bilirubin obtained from Wilson Laboratories, Chicago, was dissolved in an alkaline solution (pH 9-10) and injected intravenously in doses of 5 and 12 mgm. per kilo.   When the smaller dose was used, the bilirubin solution was injected into the femoral vein within one minute, whereas with the larger dose, five minutes were allowed for injection.   Two concentrations of sodium dehydrocholate solution were used, a 1 per cent and a 10 per cent solution.   They were injected into the blood stream through the femoral vein by means of a Woodyatt pump at a constant rate of 1 cc. per minute.   Thus, when the 10 per cent solution of dehydrocholate was injected the animal received 6.0 grams per hour and when the 1 per cent solution was injected 0.6 gram per hour.   This latter dose is more "physiological," since it represents the hourly output when the animal is producing 7.2 grams of cholates daily.

After the operation the bile was allowed to flow for approximately 1.5 to 2 hours until the volume and pigment output became constant.   A half-hour control bile flow was collected and the bilirubin solution was injected and the bile collected every 0.5 hour for a total duration of 3 hours. Samples of blood were withdrawn at 15, 30, 60 minutes and in some cases at 180 minutes.   The bilirubin determinations were made on the blood and bile by the Thannhauser and Anderson (10) modification of the Van den Bergh procedure.   In the experiments in which whole dog bile was injected, the total pigment in the bile was determined by the method of Schmidt, Jones and Ivy (11) and the bilirubin content by the Thannhauser and Anderson method (10).   Sterile gall bladder and hepatic bile were mixed and the total pigment, bilirubin and cholic acid content was determined before injection.   The whole bile was injected intravenously into the femoral vein by means of a syringe at a very slow rate so that the total period of injection took 15 minutes.   An amount of whole bile containing 5 mgm. of bilirubin per kilo was administered.   The blood pressure varied considerably during the injection and immediately following; the dose was above the intravenous bile-salt tolerance of several dogs.   The whole bile that was injected contained from 200 to 900 mgm. of cholic acid, and when 1 per cent Decholin was injected at a constant rate of 1 cc. per minute, 800 to 1500 mgm. of "cholic acid" were injected during the first hour.

RESULTS.   *Injection of 5 mgm. per Kilo of Bilirubin; Kidney Pedicles not Ligated. Without 10 per cent sodium dehydrocholate.*   The results of a control experiment on 5 dogs are shown in table 1A.   In the column labeled "extra" output are the milligrams of pigment excreted during 3 hours in excess of the control output calculated for 3 hours.   It is to be noted that the five dogs excreted 39.4 per cent of the injected bilirubin in 3 hours and 29 per cent in 1.5 hours.   The maximum recovery occurred during the second half-hour period.

With 10 per cent sodium dehydrocholate. The results of injecting 5 mgm. per kilo of bilirubin during hydrocholeresis provoked by 10 per cent sodium dehydrocholate is shown in table 1B. It is to be noted that 37.7 per cent of the bilirubin was excreted in the bile in 3 hours and 29.4 per cent in 1.5 hours.

Comment. These results show that hydrocholeresis provoked by the injection of 10 per cent dehydrocholate solution did not alter the rate of

TABLE 1A

Intravenous injection of 5 mgm. per kilo of bilirubin (kidney pedicles not ligated)

REGIME	DOG NUM-BERS	WEIGHTS	LENGTH OF EX-PERI-MENT	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RE-COVERED	PER CENT RE-COVERED IN 1½ HOURS	PER CENT RECOVERED DURING		
								½ hr.	1 hr.	1½ hr.
		kgm.	hours	mgm.	mgm.					
Control	1	17.0	3	84.0	33.89	40.3	32.0	9	14	9
	2	16.0	3	80.5	35.26	43.8	24.0	5	9	10
	3	14.0	3	70.5	26.35	37.3	30.0	5	15	10
	4	14.5	3	72.5	31.91	44.0	33.0	11	13	9
	5	13.6	3	68.0	20.84	30.6	25.0	9	9	7
Average.......	5	15	3	75.1	29.65	39.4	29.0	8	12	9

1B

Injection of 10 per cent decholin and 5 mgm. of bilirubin per kilo (kidney pedicles not ligated)

	DOG NUM-BERS	WEIGHTS	LENGTH OF EX-PERI-MENT	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RE-COVERED	PER CENT RE-COVERED IN 1½ HOURS	½ hr.	1 hr.	1½ hr.
10 per cent de-cholin (1 cc./ min.)	1	14.3	3	71.5	26.25	36.8	32.0	12	13	7
	2	18.0	3	90.0	31.40	34.9	25.0	10	9	6
	3	12.3	3	61.0	20.39	33.4	33.0	14	10	9
	4	13.0	3	65.0	16.05	24.7	22.0	9	7	6
	5	21.3	3	106.0	51.08	48.2	33.0	11	12	10
	6	11.0	3	54.5	19.19	35.2	28.0	12	10	6
	7	13.2	3	66.0	32.03	48.5	37.0	12	14	11
	8	11.4	3	57.0	18.80	33.0	25.0	12	9	4
Average.......	8	14.3	3	71.4	26.89	37.7	29.4	12	10	7

Under anesthesia when 5 mgm. per kilo of bilirubin are injected, the plasma bilirubin is 2 mgm. per 100 cc. of plasma at the end of 1 hour.

excretion of the injected bilirubin. One difference was noted, however; the maximum excretion of bilirubin in the bile occurred in the second half-hour period when no bile salt was given and in the first half-hour period when the bile salt was given (table 1).

Since only about 38 per cent of the intravenously injected bilirubin was recovered in the bile in 3 hours, it was decided to ascertain the effect of removal of the kidneys on the excretion of bilirubin in the bile. Also, since none of these animals developed visible icterus and the plasma bilirubin

concentration at 1 hour is only about 2 mgm. per 100 cc. of plasma, it was decided to inject a larger quantity of bilirubin. So in the following experiments the kidney pedicles were ligated and 12 mgm. per kilo of bilirubin were injected. It was thought that with this higher plasma content of bilirubin, choleresis might have an effect on the rate of excretion of the pigment.

Injection of 12 mgm. per Kilo of Bilirubin; Kidney Pedicles Ligated. Without 10 per cent sodium dehydrocholate. In 9 dogs 12 mgm. per kilo of bilirubin were injected and no dehydrocholate was given. The results are shown in table 2A. About 36 per cent of the injected bilirubin was excreted in the bile in 3 hours and 25 per cent in 1.5 hours. The maximum excretion occurred in the second half-hour period as when 5 mgm. per kilo of the pigment was injected. At the end of 1 hour the plasma still contained 6.2 mgm. of the pigment per 100 cc. The animals did not become visibly icteric at 3 hours.

It is interesting that the per cent of bilirubin excreted was approximately the same as when 5 mgm. per kilo was injected.

With 10 per cent sodium dehydrocholate. In 5 dogs 12 mgm. per kilo of bilirubin were injected with 10 per cent dehydrocholate. The dehydrocholate reduced the rate of excretion of the injected bilirubin, since only 14.5 per cent was recovered in the bile in 3 hours and 9.0 per cent in 1.5 hours (table 2B). At the end of one hour the amount of bilirubin in the plasma was about the same as when dehydrocholate was not given.

All of these dogs developed visible icterus. Either the dehydrocholate affected the permeability of the capillaries, so that the *indirect*-reacting bilirubin injected passed into the tissues in sufficient quantity to stain the tissues, or some of the indirect bilirubin was changed to *direct*-reacting bilirubin which passed into the tissues. At any rate so much bilirubin entered the tissues that the excretion of the pigment in the bile was decreased 2.7 times during a 3 hour period.

Comment. When 10 per cent sodium dehydrocholate is injected at 1 cc. per minute the animals receive about 6 grams per hour. This is a large dose, perhaps large enough to injure the capillaries. A dose of sodium dehydrocholate was then selected which was considered to be more "physiological", or one (1 per cent solution) that approximates the average hourly 24 hour absorption of natural cholates from the intestine, namely, 0.6 gram per hour. This dose still caused a brisk choleresis.

With 1 per cent sodium dehydrochloate. One per cent sodium dehydrocholate were used and 12 mgm. per kilo of bilirubin were given. The results are shown in table 2C. In this experiment about 41 per cent of the bilirubin was excreted in the bile in 3 hours and 24 per cent in 1.5 hours. Thus, choleresis again did not significantly augment the rate of excretion of bilirubin. Though the bilirubin concentration in the plasma was about

the same as with 10 per cent dehydrocholate administration, no visible jaundice occurred.

TABLE 2A

Intravenous injection of 12 mgm. of bilirubin per kilo (kidney pedicles ligated)

REGIME	DOG NUM-BERS	WEIGHTS	LENGTH OF EX-PERI-MENT	INTAKE	"EX-TRA" OUTPUT	TOTAL PER CENT RECOV-ERED	PER CENT RECOV-ERED 1½ HOURS	PER CENT RECOVERED DURING			BLOOD PLASMA BILIRUBIN MGM./100 CC.		
								½ hr.	1 hr.	1½ hr.	15	30	60 min.
		kgm.	hours	mgm.	mgm.								
Control	1	8.0	3	96.0	36.01	37.5	26	4	13	9			
	2	18.0	3	216.0	65.52	30.3	18	1	9	8			
	3	18.0	3	216.0	64.33	30.0	18	1	8	9			
	4	14.0	3	168.0	60.66	36.1	27	2	15	10			
	5	11.0	3	132.0	44.34	33.5	21	3	11	7	10.0	6.2	5.1
	6	12.0	3	144.0	43.02	30.0	18	2	9	7	11.1	10.0	6.2
	7	10.0	3	120.0	45.07	37.5	22	1	11	10	10.4	10.0	9.8
	8	9.0	3	108.0	34.82	30.7	19	7	5	7	8.8	8.3	6.1
	9	10.0	3	120.0	52.99	44.1	27	9	9	9	10.7	7.9	4.1
Average	9	12.0	3	146.6	49.64	33.8	21.8	3	10	9	10.2	8.5	6.2

2B

Injection of 10 per cent decholin and 12 mgm. of bilirubin per kilo (kidney pedicles ligated)

10 per cent de-cholin (1 cc./min.)	1	14.0	3	165	30.08	18.0	10	2	4	4			
	2	14.0	3	168	26.34	15.7	11	3	4	4			
	3	13.0	3	156	15.51	9.9	8	2	3	3			
	4	13.0	3	156	14.46	9.3	8	2	3	3	10.0	9.1	7.9
	5	17.0	3	204	37.49	18.3	11	3	4	4	10.0	8.8	6.9
Average	5	14.0	3	170.0	24.78	14.5	9.0	2	4	3	10.0	8.9	7.4

2C

Injection of 1 per cent decholin and 12 mgm. of bilirubin per kilo (kidney pedicles ligated)

1 per cent de-cholin (1 cc./min.)	1	18.0	3	216	98.00	45.3	30	11	10	9	12.5	11.1	7.5
	2	10.0	3	120	51.32	42.7	23	0	13	10	8.3	7.0	5.4
	3	8.0	3	96	27.00	28.1	16	5	6	5	8.1	7.5	6.1
	4	11.0	3	132	54.53	41.3	25	7	10	8	9.3		7.0
	5	12.0	3	144	63.76	44.2	28	10	9	9	13.0	10.7	9.3
Average	5	11.8	3	141.6	58.92	41.6	24.4	6	10	8	10.2	9.0	7.0

Comment. Obviously in these experiments the dose of sodium dehydrocholate determined the amount of the injected pigment which was excreted in the bile. With 10 per cent dehydrocholate the tissues became visibly stained and less pigment was excreted in the bile. With 1 per cent de-

dehydrocholate the tissues did not become visibly stained and about the same amount of pigment was excreted in the bile as in the controls which received no bile salt. However, the amount of pigment is also a factor in determining the development of icterus because 10 per cent dehydrocholate did not cause visible icterus of the tissues when 5 instead of 12 mgm. per kilo of bilirubin were injected. Since visible icterus of the tissues was present or absent with approximately the same plasma levels of bilirubin 1 hour after injection, it is possible that, when the plasma bilirubin was high at the start as with a 12 mgm. injection and the concentration of dehydrocholate was also high (10 per cent solution), some of the pigment was converted into the more diffusible direct-reacting pigment. This would account for the visible icterus and the decreased excretion of pigment in the bile when 12 mgm. per kilo of pigment were injected in association with 10 per cent sodium dehydrocholate. However, it seems more probable that some ratio between indirect-reacting bilirubin and capillary permeability or a threshold exists.

The Rate of Excretion of Pigment Injected in the Form of Whole Dog Bile; Kidney Pedicles Ligated. Since the solubility of bilirubin isolated from bile by chemical procedures is different from the solubility of bilirubin in bile, it was considered important to determine the rate of excretion of the pigment as it occurs in bile. Because there are other pigments in bile than bilirubin, both the total pigment and bilirubin were determined.

Without 1 per cent sodium dehydrocholate. In 5 dogs 5 mgm. of bilirubin per kilo body weight of dog gall bladder bile and hepatic bile were injected. The results are shown in table 3A and 3C. Approximately 9 per cent and 11 per cent of the bilirubin and total pigment respectively were recovered during 3 hours, and in each case 6 per cent was recovered during the first 1.5 hours. In all cases bilirubin was present in the plasma after 3 hours and averaged 3.2 mgm. per 100 cc. of plasma.

With 1 per cent sodium dehydrocholate. In 5 dogs 1 per cent sodium dehydrocholate was injected with whole dog bile. About 10 per cent of the bilirubin and 12 per cent of the total pigment was recovered in 3 hours and 7 per cent in 1.5 hours (table 3B and 3D). At the end of 3 hours the plasma bilirubin was approximately 2.4 mgm. per 100 cc., which was about the same as when dehydrocholate was not given.

Comment. When whole dog bile and 1 per cent sodium dehydrocholate were injected, the amount of bilirubin recovered was about the same as that recovered when 12 mgm. of bilirubin and 10 per cent dehydrocholate were injected, i.e., 10 per cent and 14.5 per cent respectively. No visible icterus developed in any of the dogs in which whole dog bile and dehydrocholate were injected. The direct-reacting bilirubin in the bile probably diffused into the tissues but the concentration of pigment was not great enough to cause visible icterus of the tissues. If 12 mgm. of pigment per kilo had

TABLE 3A

Intravenous injection of 5 mgm. per kilo of bilirubin as contained in whole dog bile

REGIME	DOG NUMBERS	WEIGHTS	LENGTH OF EXPERIMENT	CHOLIC ACID IN DOG BILE	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RECOVERED	PER CENT RECOVERED 1.5 HOURS	PER CENT RECOVERED DURING			BLOOD PLASMA BILIRUBIN CONC., MGM./100 CC.			
									½ hr.	1 hr.	1½ hr.	15	30	60	180 min.
		kgm.	hours	mgm.	mgm.	mgm.									
Control	1	9	3	820	43.7	3.98	9	4	1	1	2	5.9	7.1		4.4
	2	10	3	902	50.6	3.47	7	4	1	0	1	9.3	9.7	6.0	3.4
	3	9	3	309	44.95	3.77	8	6	4	1	1		9.7	6.5	3.0
	4	7	3	245	34.88	3.81	11	8	1	4	3	8.4	8.7	5.0	2.6
	5	8	3	450	39.95	4.07	10	7	2	3	2	9.8	8.7	5.0	2.4
Average	5	8.6	3	545	42.81	3.82	9	6	2	2	2	8.2	8.8	5.6	3.2

3B

Intravenous injection of 5 mgm. per kilo of bilirubin (whole dog bile) and 1 per cent decholin

REGIME	DOG	WEIGHT	LENGTH	CHOLIC	INTAKE	"EXTRA" OUTPUT	TOTAL	1.5 HR	½ hr.	1 hr.	1½ hr.	15	30	60	180 min.
1 per cent	1	9	3	309	44.95	6.19	13	8	3	3	2				
decholin	2	9	3	309	44.95	6.77	15	9	4	3	2	8.7	8.2	5.0	2.4
(1 cc./	3	8	3	600	40.00	2.49	6	3	0	2	1	9.0	8.5	5.4	3.0
min.)	4	11	3	690	54.81	3.61	6	2	0	1	1	8.7	8.0	5.4	2.4
	5	8	3	510	39.95	3.87	9	6	2	3	1	9.0	8.4	4.4	2.0
Average	5	9	3	483	44.93	4.59	10	6	2	3	1	8.8	8.3	5.0	2.4

3C

Recovery in the bile of "total pigment" after intravenous injection of dog bile

REGIME	DOG	WEIGHT	LENGTH	CHOLIC	INTAKE	"EXTRA" OUTPUT	TOTAL	1.5 HR	½ hr.	1 hr.	1½ hr.
Control	1	9	3	820	107.2	12.42	12	4	1	1	2
	2	10	3	902	117.9	12.74	6	2	0	1	1
	3	9	3	309	112.3	21.10	18	13	7	2	3
	4	7	3	245	87.2	6.12	7	4	2	1	1
	5	8	3	450	98.4	8.45	8	5	2	2	1
Average	5	8.6	3	545	104.6	12.16	11	6	3	1	2

3D

Recovery in the bile of "total pigment" after intravenous injection of dog bile and 1 per cent decholin

REGIME	DOG	WEIGHT	LENGTH	CHOLIC	INTAKE	"EXTRA" OUTPUT	TOTAL	1.5 HR	½ hr.	1 hr.	1½ hr.
1 per cent	1	9	3	309	112.3	16.05	14	9	3	4	2
decholin	2	9	3	309	112.3	17.80	16	9	3	3	3
(1 cc./	3	8	3	600	120.0	15.65	13	8	3	3	2
min.)	4	11	3	690	164.4	14.57	9	6	2	3	1
	5	8	3	510	120.0	13.60	11	8	3	4	1
Average	5	9	3	483	125.8	15.53	12	8	3	3	2

been injected instead of 5 mgm., visible icterus probably would have resulted.

Because of the toxicity of whole bile this experiment is more pathological than physiological in type. However, if it is essential to use a bilirubin with the same solubility as that in bile, bile must be used, at least for the present.

Bilirubin Accounted for at the End of One Hour. The total pigment accounted for at the end of 1 hour may be calculated by adding that recovered in the bile to that still present in the blood plasma. The remainder must be in the cells or in the body fluids other than plasma in those experiments in which the kidneys were excluded. When 5 mgm. per kilo of bilirubin were injected without kidney exclusion and dehydrocholate, 34 per cent of the pigment was in the bile and blood plasma at one hour; when the same amount was injected with 10 per cent dehydrocholate 37 per cent was in the bile and blood plasma. When 12 mgm. per kilo of bilirubin were injected with kidney exclusion but without dehydrocholate 39 per cent was in the bile and blood plasma; under similar conditions but with 1 per cent dehydrocholate, 39 per cent of the pigment was in the bile and blood plasma, but with 10 per cent dehydrocholate only 30 per cent was in the bile and blood plasma. Since the latter dogs developed jaundice the difference between 39 and 30 per cent, or about 15 mgm. of pigment, represented a sufficient difference to cause visible tinting of the tissue.

It was noted that after a 3-hour period the rate of excretion was quite low regardless of the fact that choleresis during the third hour was the same as during the preceding hours. This shows that the rate of excretion of the bilirubin remaining in the body occurs at a slow rate. The time for complete removal will have to be determined on dogs with a permanent bile fistula.

GENERAL DISCUSSION. On the basis of the data obtained it is clear that choleresis induced by the intravenous injection of sodium dehydrocholate, a pure and relatively non-toxic choleretic agent, does not augment significantly the rate of excretion of intravenously injected bilirubin in the anesthetized dog. This result, however, does not necessarily indicate that the administration of sodium dehydrocholate, or a similarly acting choleretic, after the relief of common-duct obstruction, will not increase the rate of excretion of bilirubin. This question can only be answered directly by performing a controlled series of experiments on a group of animals in which jaundice is produced by obstruction of the common bile duct and then the obstruction released.

SUMMARY

Using anesthetized dogs with exclusion of the gall bladder and the common duct cannulated for the collection of bile, it was found that the

production of choleresis by the continuous intravenous injection of sodium dehydrocholate (0.6 or 6.0 grams per hour) does not increase the rate of removal of intravenously injected bilirubin (5 or 12 mgm. per kilo body weight) from the blood stream or its excretion in the bile. This is also true when whole dog bile is used as a source of bile pigment. When 12 mgm. of bilirubin per kilo body weight are injected, or the bilirubin level of the blood is elevated to 9 to 13 mgm. per 100 cc. of plasma, the injection of an excessive amount of sodium dehydrocholate (6.0 grams per hour in our experiments) converts a hyperbilirubinemia without jaundice into a hyperbilirubinemia with jaundice. These results do not necessarily indicate that the administration of sodium dehydrocholate, or a similarly acting choleretic, after the relief of common-duct obstruction will not increase the rate of excretion of bilirubin and the disappearance of jaundice.

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THE EFFECTS OF ESTROGEN AND THYROIDECTOMY IN FEMALE RATS ON THE EXCRETION OF CREATINE AND CREATININE[1]

JAMES B. ALLISON AND SAMUEL L. LEONARD

From the Laboratories of Physiology and Biochemistry and of Zoology, Rutgers University, New Brunswick, New Jersey

Among the various factors influencing creatinine and creatine excretion in mammals, the species, sex, age, diet and the hormonal state of the body are of outstanding importance. Both adult male and female rats normally excrete creatine when fed on diets containing creatine or creatine precursors, such as those which include meat scrap. Injection of thyrotropic hormone or thyroxin increases the excretion of creatine in rats (Pugsley, Anderson and Collip, 1934). On the other hand, thyroidectomy decreases and subsequent injections of thyroxin increase the excretion of creatine in male rats on a diet containing creatine or creatine precursors (Allison, Glaser and Leonard, 1939). Kun and Peczenik (1935) report that in female rats during diestrum or after castration, the creatine excretion is either low or lacking, whereas during estrum or after the administration of estrogens, the creatine excretion rises markedly. Beard and Jacob (1940b) report that castration in the male and female rat is followed by a temporary creatinuria (due to traumatic injury?) and that injection of sex hormones (estrogen, male hormone) induces creatinuria in recently castrated rats, but not in those deprived of their gonads for 90 days. The present investigation was undertaken to determine in female rats, fed a diet producing a marked creatinuria, 1, the effect of the estrous cycle; 2, of castration and thyroidectomy, and 3, of estrogen injections on the excretion of creatinine and creatine.

MATERIALS AND METHODS. The rats used in these experiments were young adult females (approximately 200 grams) of the Long-Evans or Langley strains. The piebald rats were raised on Purina dog chow, but throughout these experiments were fed Russell's diet BMS (1932), which is a modified Sherman diet B containing meat scrap. The Langley rats

were raised on diet BMS. They were placed in individual metabolism cages several days before urine collections were started to acclimate them to their new surroundings. Water and food were provided ad lib. The 24-hour urine samples, free from feces and food particles, were collected under toluene. The screen bottom of each cage and collecting funnel were washed with distilled water. The washings were combined with the urine sample and the volume made up to 100 ml. The body weights of the rats were measured at the time of the urine collection. The micromethods of Folin (1914) were used for the determinations of creatinine and creatine, autoclaving to convert the latter substance into creatinine. The results are expressed in millimols of creatinine or of creatine excreted in 24 hours per kilogram of body weight.

RESULTS. *Normal excretion.* Four female rats of the piebald strain (170-177 grams) were placed in the metabolism cages for a period of one month and occasional determinations of creatinine and creatine were made (15-24 hr. samples). For the next 27 days, daily determinations were made and the estrous cycles were followed by the vaginal smear method. These data are illustrated by circles in figure 1, where millimols of creatine are plotted against millimols of creatinine per kilogram of body weight. The averages for these 164 determinations, with the probable errors, are 0.302 ± 0.0023 millimol of creatinine and 0.163 ± 0.0032 millimol of creatine. At the end of these experiments the rats had gained an average of 32 grams. The millimols of creatinine excreted are distributed around the mean, which is independent of the magnitude of the excretion of creatine.

No correlation was found between the stages of the estrous cycle and the amount of excretion of creatine or of creatinine. The daily ratios of creatine to creatinine are plotted in figure 2 against the days of collection of the urine. The periods of heat occurred some time during the 24 hours represented by circles with crossed lines. The ratios were used, instead of absolute values, to illustrate variation in the excretion of creatine because they are not affected by small losses in urine which might occur during the collection of 24 hour samples. These data demonstrate that there is no marked effect of the period of heat upon these ratios.

Castration. These rats were castrated and after a period of 15 days daily determinations were made of creatine and creatinine for 17 days. These data are illustrated in figure 1 by the closed squares. The averages of the 68 determinations, with the probable errors, are 0.295 ± 0.0037 millimol of creatinine and 0.100 ± 0.0043 millimol of creatine per kilogram of body weight. There is no change from normal in the excretion of creatinine, but there is a drop in the excretion of creatine in these castrated females. For a statistical comparison, nine consecutive daily determinations of creatine in normal and castrated female rats were selected. Based
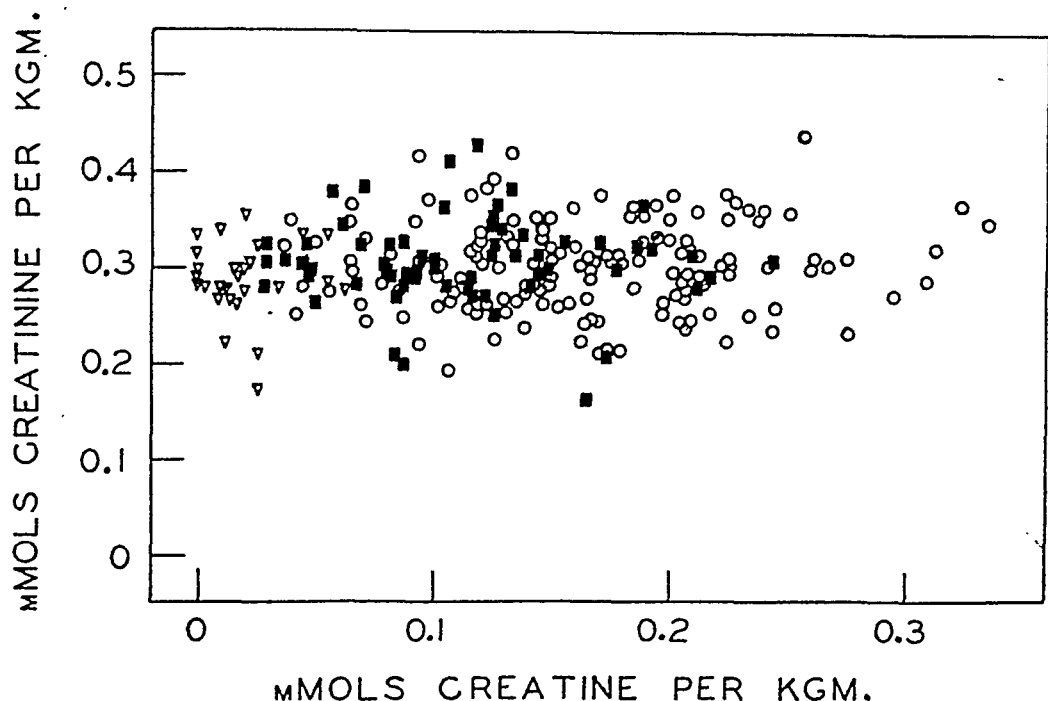
Fig. 1. The millimols of creatine excreted per kilogram of body weight during the 24 hours by four female rats, are plotted against the millimols of creatinine per kilogram excreted over the same period of time. The circles represent excretion in the normal, the closed squares excretion in the castrated and inverted triangles the excretion in the castrated, thyroidectomized female rats.
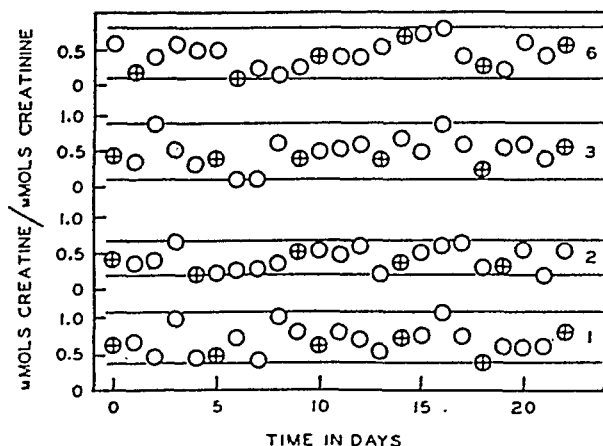
Fig. 2. The ratios between the millimols of creatine and creatinine excreted, are plotted against time in days. The data for rats 1, 2, 3 and 6 are plotted separately and the circles with crossed lines represent the ratios obtained during the 24 hour period when the rats were in heat.

on millimols of creatine excreted per kilogram of body weight, the drop in the excretion of creatine in castrated female rats is significant since $P = 0.05$ by Fischer's method.

Thyroidectomy. The thyroid glands (thyro-parathyroidectomy) were removed from the four piebald rats and after a period of eight days, determinations were made during nine consecutive days. These data illustrated in figure 1 by the inverted triangles, indicate that thyroidectomy markedly lowers the excretion of creatine without affecting the excretion of creatinine. The averages for 34 determinations, with the probable errors, are 0.291 ± 0.004 millimol of creatinine and 0.022 ± 0.0002 millimol of creatine excreted per kilogram of body weight.   .

A similar decrease in the excretion of creatine was found in females of the Langley strain, when they were thyroidectomized but not castrated. The mean excretion of millimols of creatine per kilogram based on 15 determinations on three normal rats was 0.109. Four other females of the same strain were thyroidectomized and after a rest period of eight days, the average millimol of creatine excreted per kilogram was 0.007 for 20 determinations. The excretion of creatinine was not altered by thyroidectomy.

Estrogen in castrated rats. Since castration lowered the excretion of creatine, it seemed important to study the effects of estrogenic hormone treatment in the castrated rats. The four castrated piebald rats, before they were thyroidectomized, were given a single injection of 60 R.U. of an estrogenic hormone (progynon B)[2]. The average excretion of creatinine and of creatine for five days preceding the injections are compared in table 1 with the average excretion of these substances for the five days following the injections. These data show a small but definite rise (0.105 to 0.134 millimol) in the excretion of creatine with no change in the excretion of creatinine following the injection of the hormone. This rise of creatine following estrogen treatment is significant ($P < 0.05$ and almost equal 0.02, Fischer's method). Included in this table are average millimols of creatinine and creatine per kilogram of body weight excreted by the four normal female rats for a period of five consecutive days taken at random from data appearing in figure 1.

The average daily excretions of creatinine and of creatine in four other castrated female rats before and after injections of 5000 R.U. of estrogen are recorded in table 2. The average excretions of creatinine and creatine during the first eight days before injection of the hormone are respectively 0.271 millimol and 0.073 millimol per kilogram of body weight. The rats were injected with 5000 R.U. of estrogen on the ninth day. The average millimols of creatine excreted per kilogram of body weight increased on the eleventh day coincidentally with the appearance of full heat in the vaginal smear. From the data presented in table 2, the average excretion of creatinine is 0.258 millimol and of creatine is 0.150 millimol per kilogram

during the six days the rats were in heat. The slightly lower average excretion of millimols of creatinine during the heat period is due to the

TABLE 1

Average millimols of creatinine and creatine per kilogram of body weight excreted daily for five days by four female rats under various experimental conditions

CONDITIONS	WEIGHT	CREATININE	CREATINE
	kgm.	*mmols/kgm.*	*mmols/kgm.*
Normal female............................	0.205	0.301	0.168
Castrated female.........................	0.252	0.305	0.105
Castrated + estrogen (60 R.U.)...........	0.242	0.294	0.134
Castrated + thyroidectomy...............	0.247	0.295	0.024
Castrated + thyroidectomy + estrogen (60 R.U.)................................	0.251	0.290	0.027
Castrated + thyroidectomy + estrogen (20,000 R.U.)............................	0.247	0.290	0.023

TABLE 2

Average millimols of creatinine and creatine per kilogram of body weight excreted for eight days by four castrated female rats before and after the injection of 5000 R.U. of estrogen

DAYS	WEIGHT	CREATININE	CREATINE
	kgm.	*mmols/kgm.*	*mmols/kgm.*
1	0.214	0.269	0.099
2	0.219	0.240	0.067
3	0.222	0.293	0.097
4	0.225	0.301	0.044
5	0.234	0.293	0.050
6	0.237	0.278	0.078
7	0.240	0.212	0.032
8	0.240	0.266	0.089
9*	0.245	0.308	0.064
10	0.235	0.257	0.083
11	0.235	0.184	0.140
12	0.227	0.255	0.176
13	0.228	0.242	0.164
14	0.226	0.300	0.145
15	0.227	0.281	0.141
16	0.226	0.286	0.135

* Injected 5000 R.U. of estrogen.

marked drop in the excretion of this substance on the eleventh day, the significance of which is not clear.

Estrogen in thyroidectomized rats. The four piebald rats, which were both thyroidectomized and castrated, were injected with 60 R.U. of the

estrogenic hormone and after a period of five days received another injection of 20,000 R.U. The results are presented in table 1. The average millimols of creatine excreted per kilogram for five consecutive days preceding the injections are 0.024, after the first injections of 60 R.U. are 0.029 and after the massive dose are 0.023. The administration of the hormone, therefore, failed to influence the creatine level of excretion. The creatinine was unchanged.

The four rats of the Langley strain, which were thyroidectomized but not castrated, were injected with 5000 R.U. of estrogen. The average excretion of creatine on the day of injection was 0.0343 millimol and the average excretion for four days following the injection was 0.030 millimol. The excretion of creatinine was not altered by the injection. These experiments with thyroidectomized rats indicate that estrogen increases the excretion of creatine only when the thyroid glands are present.

DISCUSSION. There was no apparent relationship between the excretion of creatine and creatinine in normal female rats exhibiting marked creatinuria. Since Block and Schoenheimer (1939), in their studies of the metabolic relationships of creatine and creatinine using isotopic nitrogen, found that body creatine is the precursor for the creatinine excreted in the urine, some relationship between the excretion of these two substances might be expected. Beard and Jacob (1940a), on the other hand, found that injection of creatine into rats did not increase the excretion of creatinine. Saturation of the body with creatine due to excess creatine or precursors in the diet of rats would explain the independence of this excretion of creatine and creatinine.

Contrary to the results of Kun and Peczenik (1935), the estrous cycle in our female rats could not be correlated with the daily fluctuations in the excretion of creatine. These fluctuations were as great in the castrated rats as in the normal rats, although at lower levels. Possibly the variations in the circulating concentration of estrogens in rats during the estrous cycle were not large enough under the conditions of our experiments to enable an effect on creatine excretion to be observed. Removal of the ovaries, for example, lowered the excretion of creatine slightly, but not to the extent reported by Kun and Peczenik. That there may be a sex difference in the effect of hypogonad function on creatinuria in the rat is suggested by the work of Coffman and Koch (1940), who state that "castration of adult male rats does not alter creatine excretion."

A marked reduction in the excretion of creatine in the female rat, as in the male rat (Allison, Glaser and Leonard, 1939), followed removal of the thyroid gland. The average excretion of creatinine by the thyroidectomized female rats remained within the range shown by the normal and castrated animals.

The increase in creatine excretion following injection of estrogen into

rats with intact thyroids is in agreement with the results of Kun and Peczenik (1935) and Pizzolato and Beard (1939). Since this response was not observed in the thyroidectomized animals, the thyroid gland may be an important intermediary organ in controlling the effect of estrogen on creatinuria. There are other evidences in the literature which support this suggestion. Anderson and Kennedy (1933), for example, have shown that gonadectomy produces atrophy of the thyroid in the mature female rat, which is most obvious eight weeks after the operation. The tendency for a lowering of the excretion of creatine, as well as the observations made by Beard and Jacob (1940b), that injection of sex hormones into 90-day castrated rats would not produce creatinuria, may be due to the atrophy of the thyroid.

There is also some evidence that the action of estrogen on thyroid activity, hence alterations in creatinuria, may occur by way of the hypophysis. Turner and Cupp (1940) have shown that gonadectomy lowers the thyrotropic hormone content of the hypophysis and that injections of estrogen maintain the normal level of thyrotropin in the castrated female hypophysis. Since it is known that thyrotropic hormone causes an increase in creatine excretion (Pugsley et al., 1934), it might be assumed that a decrease in the output of this hormone following castration would result in a lowered excretion of creatine. Furthermore, the administration of estrogens into castrated rats did not raise the level of excretion of creatine above normal in our experiments nor did they increase the thyrotropic hormone content of the hypophysis above normal in the experiments of Turner and Cupp. It is possible, therefore, that changes in creatine excretion, which result from female sex hormone imbalance of the body, may be due to alteration in thyroid function as mediated by the hypophysis.

SUMMARY

The data presented demonstrate 1, that the average excretion of creatinine remains constant in these normal, castrated, and thyroidectomized female rats, fed on a diet containing meat scraps; 2, that there is no apparent correlation between the estrous cycle and the excretion of creatine; 3, that castration lowers slightly the excretion of creatine; 4, that thyroidectomy lowers this excretion markedly, and 5, that estrogen increases the excretion of creatine in castrated rats, but has no effect upon the excretion of creatine in thyroidectomized rats.

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THE METABOLISM OF HUMAN SPERMATOZOA[1]

JOHN MacLEOD

From the Department of Anatomy, Cornell University Medical College,
New York, N. Y.

In a preliminary communication (8) the metabolism of human spermatozoa in Ringer-glucose was briefly described. It was shown that the metabolism was almost exclusively glycolytic. The present report will deal in greater detail with the metabolic behavior of these cells.

The available information on the metabolism of human spermatozoa is scanty, and in the main is confined to data derived from incubation of semen (3, 6, 7). Recently, Shettles measured respiration and respiratory quotients using undiluted semen. Since Shettles' paper raises fundamental questions of technique, discussion of his results will be considered later.

Before presenting the present results, it is necessary to mention certain points about the use of Ringer-glucose as a medium. Since most measurements of tissue metabolism are made in an artificial medium such as Ringer's solution, it was considered desirable for purposes of comparison, to obtain such data for the spermatozoa. Furthermore, the use of Ringer-glucose has certain obvious advantages in that when lactic acid production in the presence of bicarbonate is measured, the CO_2 of glycolysis can be determined directly since Ringer's is a non-retentive medium. On the other hand, there are often objections to the use of artificial media, and as is well known tissues bathed in blood serum may show a greater and longer sustained metabolic activity than the same tissues in phosphate or Ringer's (15, 9). These authors have emphasized the technical difficulties inherent in the use of serum, these being, principally, the problems of measuring respiration *directly* in the presence of the serum bicarbonate and of allowing for the high CO_2 retention which is a characteristic of blood serum. One is faced with the same problems in the choice of a physiological medium for spermatozoa. Seminal fluid contains bicarbonate in about the same concentration as blood serum (3) so that direct measurements of O_2 consumption in the presence of KOH cannot be made. Furthermore, seminal fluid, like blood serum, "binds" CO_2 to a marked degree so that the manometric measurements of the CO_2 liberated from bicarbonate by

lactic acid cannot be made without the use of CO_2 retention curves and other technical refinements.

As a final consideration in the choice of Ringer-glucose, the viability of the cells is of prime importance: Spermatozoa, considered as a tissue, are unique in that motility may be used as a criterion of viability. In all of these experiments, we have seldom met with an instance in which the motility of the cells after many hours in Ringer-glucose at 38°C. showed any decrease compared with the motility of the same cells in seminal fluid under the same experimental conditions. These points will be considered in greater detail under separate headings in the body of the paper.

METHODS. The technique used was that of Warburg (14), and all results obtained manometrically except in a few cases where chemical determinations of lactic acid were made to check the manometric results.[2] The spermatozoa were obtained from young, healthy adults at intervals of four or five days and were received in the laboratory within an hour after ejaculation. The semen was then centrifuged, the seminal fluid decanted, and the cells transferred to Ringer-glucose. After the number of cells per cubic centimeter was determined and motility examined, the cells were transferred to the manometer vessels. Respiration was measured in phosphate (pH 7.4) by the direct method of Warburg (14) and in a bicarbonate medium by the use of the Summerson differential manometer (13). For measurement of glycolysis, bicarbonate was added to make a final concentration of 0.03M and the system equilibrated with gas mixtures containing 95 per cent O_2 and 5 per cent CO_2 and 95 per cent N_2 and 5 per cent CO_2. All measurements were made in duplicate and manometric readings made every 30 minutes. The experimental periods ranged from 3 to 10 hours. The results given below represent the mean metabolism per hour during the first 3 hours.

OBSERVATIONS. I. *Glycolysis.* Since glycolysis is the outstanding feature (8) of the metabolism of human spermatozoa, this will be considered first. Table 1 gives figures for aerobic and anaerobic lactic acid formation. The results are expressed as mm.[3] $CO_2/10^8$ cells/hour and, in more conventional fashion, as Q_G or the amount of CO_2 per mgm. of dry tissue per hour.[3]

The glycolysis figures are presented in two groups (table 1), the first representing the metabolism of 83 individual specimens and the second, 71

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[2] In the latter experiments, the method of Friedman, Cotonio, and Shaffer (11) was used and these chemical determinations were found to check the manometric reading within ±8 per cent. I am indebted to Dr. S. B. Barker for making these determinations.

[3] Previously, these results were presented as mm³$CO_2/10^6$ cells/hour. It was thought advisable here to use the higher number of cells so as to allow the use of whole numbers.

experiments in which two or more specimens were combined.   The two groups are not combined since the standard error of the difference between the means shows that the glycolysis of the combined specimens is significantly higher than that of the individual specimens.   The 83 experiments on individuals represent material from only 30 men, with either one or several specimens from each individual.

Table 2 represents figures obtained from the spermatozoa of 5 individuals at different times.   It will be seen that while there are striking differences

TABLE 1

Mean anaerobic and aerobic glycolysis of individual and combined specimens

NUMBER OF EXPERIMENTS	ANAEROBIC GLYCOLYSIS					AEROBIC GLYCOLYSIS				
	mm.^{3} CO_2/10^3 cells/ hour	High	Low	σ	Mean Q^{N_2}G	mm.^{3} CO_2/10^3 cells/ hour	High	Low	σ	Mean Q^{O_2}G
Individual 83...	10.6	27.2	3.5	4.8	7.4	8.5	22.8	3.2	4.0	5.9
Combined 71 ...	12.1	24.6	3.2	3.9	8.4	10.4	22.0	3.2	3.9	7.2

TABLE 2

Anaerobic and aerobic glycolysis of individual specimens

DATE	NO. 1E mm.^{3} CO_2/10^3 CELLS/HOUR		DATE	NO. 2C mm.^{3} CO_2/10^3 CELLS/HOUR		DATE	NO. 3L mm.^{3} CO_2/10^3 CELLS/HOUR		DATE	NO. 4K mm.^{3} CO_2/10^3 CELLS/HOURS		DATE	NO. 5T mm.^{3} CO_2/10^3 CELLS/HOUR	
	N$_2$	O$_2$		N$_2$	O$_2$		N$_2$	O$_2$		N$_2$	O$_2$		N$_2$	O$_2$
2/24/39	12.1	10.7	3/27/39	7.1	6.8	2/15/40	24.5	22.8	4/ 5/39	15.9	15.9	3/31/39	4.9	5.0
3/14/39	7.4	7.2	3/31/39	8.1	7.0	2/19 40	24.3	21.6	4/ 8/39	11.2	10.0	4/ 7/39	4.9	4.0
3/29/39	10.0	8.5	4/ 5/39	6.6	6.6	2/23/40	27.2	22.0	4/18/39	10.3	8.0	4/14/39	3.5	3.2
4/ 5/39	11.0	9.2	4/11/39	13.2	12.5	2/29/40	20.6	19.0	4/26/39	13.4	12.6	4/28/39	5.6	4.0
4/26/39	8.5	7.9	4/19/39	7.5	7.0	3/ 4/40	28.0	20.0	4/29/39	11.7	9.0	5/31/39	10.0	8.0
6/ 6/39	11.4	11.4	4/25/39	10.1	9.6				5/24/39	9.7	7.3	9/22/39	7.7	6.0
			6/ 2/39	7.5	7.5									
			6/22/39	8.5	7.6									

in lactic acid production between individuals, the acid production of the sperm from any one remains relatively constant from week to week. Sharp rises or falls may occur from time to time which cannot be accounted for on the basis of motility since all of the specimens showed maximal and sustained motility.   Whether the glycolysis is high or low, the ratio between aerobic and anaerobic glycolysis remains relatively constant, though in several instances there was no evident difference between aerobic and anaerobic metabolism.

There are striking differences in the glycolysis of spermatozoa of different

individuals.   This is most apparent if the figures for 3L and 5T are compared.   The acid production of 3L is about four times that of 5T and it is obvious that the glycolysis of one does not tend to approach that of the other even when one is high and the other low.   Again, it should be emphasized that motility is not a factor as the sperm of both individuals showed maximal motility at all times.[4]   In regard to cell morphology this factor can be dismissed briefly; no significant difference was found in any specimen throughout the course of these experiments.   There is no evidence at present to explain the quantitative metabolic differences between specimens
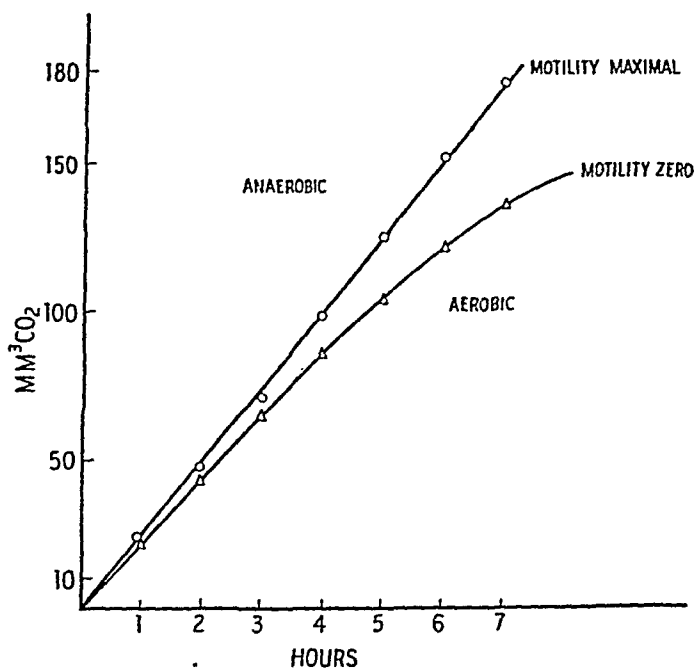
Fig. 1. Anaerobic and aerobic glycolysis of human spermatozoa in Ringer-glucose-bicarbonate.   Number of cells present = 270 million.   The motilities represented in the figure were taken at the end of the 7-hour period.

of different individuals and those which may be found from time to time in the same individual.

The relatively small difference between anaerobic and aerobic glycolysis (15–20 per cent) is striking when one considers that aerobic glycolysis, when present in normal adult tissues, is usually of small magnitude and never approaches the magnitude of the anaerobic metabolism.

Figure 1 shows an experiment in which aerobic and anaerobic glycolysis was measured on specimen 2C over a period of seven hours.   This experiment is selected because it demonstrates attributes common to many

[4] In a study of sheep spermatozoa, Comstock (1) has found significant differences in glycolysis between semen of different rams, these differences being independent of motility and sperm numbers.

specimens of human spermatozoa. Anaerobic glycolysis is linear over the entire period while aerobic glycolysis tends to fall off slightly with time. This decrease in *aerobic* glycolysis becomes apparent, in most specimens, only after several hours, and is coincidental with a failure of motility. Human spermatozoa, under certain experimental conditions, are *sensitive to injury* in the presence of O_2 as evidenced by failure of motility and of lactic acid production. Figure 1 is a striking example of this phenomenon. At the end of the 7 hour experimental period, under identical conditions, motility in N_2 was maximal whereas motility in O_2 was zero. This effect in O_2 does not appear in every experiment because few of the experiments reported here lasted longer than 5 hours and a time factor is involved. In other words, the "O_2 effect" is not always an "all or none" phenomenon. Usually, it appears after the cells have been in air or O_2 for several hours at 38°C. If the motility in N_2 and O_2 be compared, no qualitative difference may be seen, but if the per cent of cells motile be calculated, the number of cells motile in O_2 is frequently sharply decreased. Thus, unless anticipated, this phenomenon may escape notice.

II. *Respiration.* The initial experiments in this research were designed to determine the minimal number of cells necessary for accurate determination of O_2 consumption and glycolysis. Concentrations of cells ranging from 50 to 300 million cells per cubic centimeter in Ringer-glucose-phosphate were set up in separate manometers for measurement of O_2 consumption by the direct method (2), 1 cc. of suspension being added to the vessels. Small vessels (8 cc. vol.) were used to obtain maximal sensitivity. Within this cell-concentration range, the O_2 consumption over a 3 hour period was so small as to appear negligible, particularly since the same cells in Ringer-glucose-bicarbonate showed an easily detectable anaerobic and aerobic lactic acid production. Thus, it was apparent that sufficient cells were present in the system to show an adequate O_2 consumption if that type of metabolism existed to any marked degree.

Since the respiration evidently was quite small and since large amounts of tissue were not available, it became necessary to work with a manometric system of greater sensitivity. The experiments were re-designed so that the 8 cc. vessels would be filled with the sperm suspension, leaving a gas phase of approximately 1.5 cc. and so increasing the sensitivity of the system about four times. It is obvious that KOH cannot readily be used in such a system for the absorption of respiratory CO_2, but since the fluid volume is large and the solubility of CO_2 is great relative to that of O_2, little CO_2 will escape into the gas phase. Under these conditions a small O_2 consumption was detectable in 25 experiments and the mean figure for the first hour is given in table 3. In 8 other experiments, no oxygen consumption was evident, although in each case the aerobic glycolysis was less than the anaerobic. Several measurements of oxygen

consumption were made in a bicarbonate medium using the differential manometer technique of Summerson (13). Continuous measurements are not possible with this technique, but the *total* O_2 consumption in bicarbonate is similar to that in phosphate. Because of the limited amount of spermatozoa available at any given time, and the exceedingly small respiration of these cells, the measurements of oxygen consumption presented here are subject to greater error than those of glycolysis.

In a series of experiments to determine if the O_2 consumption was cyanide sensitive, the results show that cyanide ($10^{-3}M$) does not inhibit O_2 consumption when the latter is detectable. Furthermore, motility is not depressed either in the presence of cyanide or of carbon monoxide. When these facts are considered with the maintenance of maximal motility under anaerobic conditions, it will be seen that the motile activity of human spermatozoa is not dependent on energy derived from respiration.

As Keilen (5) and others have shown, all actively respiring tissues, when examined spectroscopically under anaerobic conditions, show the absorption spectrum of reduced cytochrome. Spectroscopic examination of a

TABLE 3

Mean oxygen consumption per hour of individual specimens

NUMBER OF EXPERIMENTS	MEAN OXYGEN CONSUMPTION PER 10^5 CELLS PER HOUR	HIGH	LOW	MEAN Q_{O_2}
25	1.8	3	0.3	1.25

concentrated suspension of human spermatozoa shows that if cytochrome is present in these cells, it does not exist to any marked degree. No bands of reduced cytochrome can be seen.[5]

The only other figures available on the respiration of *human* spermatozoa are those given by Shettles (12) who measured the respiration and R.Q. in *semen*. His figures are about 7 times higher than the highest figures reported here, and it is rather difficult to account for such a large discrepancy solely on the basis of the cells being in seminal fluid in the one experiment and in Ringer-glucose in the other. However, on the basis of the use of untreated semen, Shettles' experiments are open to question on several counts. He used the *direct* method of Warburg in measuring respiration and the *first* method of Dickens and Simer in measuring respiratory quotients. These methods are described in detail by Dixon (2). As Dixon points out, these methods give reliable results only when the medium is buffered with phosphate and are subject to severe limitations when bicarbonate is present in the medium. Seminal fluid contains large

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[5] I am indebted to Dr. Dean Burk for the use of his spectroscopic equipment and for much helpful advice in certain of the techniques used in this work.

amounts of bicarbonate and when, as in the direct method of Warburg it cannot be equilibrated with CO_2, the pH of the system shifts progressively to the alkaline side due to loss of CO_2. The measurement of respiratory quotients by the method used by Shettles is liable to serious inaccuracies because the presence of bicarbonate gives a value for preformed CO_2 which is high relative to respiratory CO_2.

However, we have repeated Shettles' experiments on the respiration of undiluted semen and are unable to confirm his results. He states that the O_2 consumption is proportional to the number of cells present and that seminal fluid devoid of spermatozoa had no O_2 consumption. We have used specimens of semen containing from 0.5 to 151 million sperm and specimens containing none. Table 4 shows the O_2 consumption of these specimens and the average O_2 consumed per 10^8 cells per hour. It will

TABLE 4

Oxygen consumption of undiluted semen

NUMBER OF CELLS IN SEMEN	OXYGEN CONSUMED PER HALF-HOUR (CU. MM.)						AVERAGE OXYGEN CONSUMED PER HOUR	AVERAGE OXYGEN CONSUMED PER 10^8 CELLS PER HOUR
	30	60	90	120	150	180		
millions								
0*	35	4.8	10	14			31.9	
0.5	34	4	3.6				26	52
3.5	14	4.8	2.4				11.6	3.3
28	40	18	8.4	8.4	6	6	28.9	1.0
70.5	31	2.4					16.7	0.23
75.5	40	2.4					21.2	0.28
131	24	12	9.6	4.8	4.8	4.8	20	1.52

* High leucocyte count.

be seen that in every case, during the first 30 minutes, there is an O_2 consumption which is high relative to that during successive 30 minute readings but that the overall O_2 consumption is in no way proportional to the number of cells present. It is likely that absorption of O_2 by the seminal fluid itself is a factor which has not properly been taken into account. The mechanism of such an absorption is not clear but it may be pointed out that cell-free blood serum also absorbs small amounts of O_2 under similar conditions (10).

III. *Substrates.* Added substrate in the form of utilizable sugar is essential for maintenance of glycolysis and of motility. Maltose, mannose, fructose and glycogen can be substituted for glucose with no evident difference in the level of glycolysis or the quality of motility, but the cells cannot utilize lactose, sucrose, or galactose. When these sugars are substituted for glucose, glycolysis and motility fail rapidly. The effective concentration of utilizable sugar lies between 20 and 200 mgm. per cent.

At high sugar levels (0.6–1.0 per cent) the spermatozoa show signs of toxicity as evidenced by a failure of motility.

IV. *The presence of bacteria.* Early in this work it became apparent that most specimens of human spermatozoa contained bacteria and that they were infected at the source, e.g., in the prostatic secretions or in the seminal vesicles. The presence of bacteria in the sperm suspensions at 38°C. usually is not manifested until between the third and sixth hours. Bacterial growth in the system shows itself by an increase in lactic acid production which proceeds logarithmically. The most persistent bacterium is a Gram-positive anaerobic streptococcus and the metabolic figures affected are those of anaerobic glycolysis. On occasion, the growth of aerobic bacteria is seen in a rapid rise in O_2 consumption. Though the kinetics of bacterial metabolism cannot be discussed at length in this paper, it can be shown that the metabolic figures for the spermatozoa are not affected by the presence of bacteria during the early hours of the experiments. This can be demonstrated briefly. We were fortunate in obtaining 3 donors whose specimens at no time showed bacterial contamination. The glycolysis figures for the sperm of these donors are represented in table 2 (2C, 1F and 5T). The figures for 2C and 1F fall within the normal metabolic range while those of 5T, on several occasions, were considerably lower. Since no bacterial growth was evident in any of these cases it is clear (a), that the mean figures for glycolysis of sterile specimens is within the limit of variation of specimens which are infected (see table 1), and (b), that wide variations in lactic acid production may exist between bacteria-free specimens of different individuals. Furthermore, it may be pointed out that the ratio between aerobic and anaerobic glycolysis during the first 3 hours in all cases is relatively constant. One would expect to find this ratio extremely unstable if bacteria contributed much acid during the early hours since bacterial growth may vary from time to time, particularly in the anaerobic system. But this ratio is quite constant during the first 3 hours.

DISCUSSION. The predominance of glycolysis and the relative deficiency of respiration in the metabolism of human spermatozoa, coupled with the fact that these cells retain maximal motility under anaerobic conditions would indicate that respiration is secondary and not an essential part of the metabolic function. Redenz (11) and Ivanow (4) come to similar conclusions for the sperm of the dog, ox and guinea pig in spite of the fact that these cells have a high respiration relative to the human. Comstock (1) also found that the respiratory activity of sheep spermatozoa is not associated with motility. In the work reported here, the *nature* of the O_2 consumption of human spermatozoa is left open for further investigation. It is not at all certain that the small O_2 consumption is operating through a cytochrome system, or indeed, that these cells possess, to any

degree, any of the hemin systems now known to be essential components of the main respiratory pathway in living cells. Analyses of the respiratory and glycolytic pathways of human spermatozoa are somewhat handicapped by the small amounts of tissue available at any given time, but investigations are in progress and will be reported later.

SUMMARY

1. The metabolism of human spermatozoa in Ringer-glucose is characterized by a high aerobic glycolysis and a relative deficiency in respiration.

2. The glycolysis of the spermatozoa of any one individual is relatively constant from specimen to specimen but large differences are found from individual to individual. These differences cannot be accounted for on the basis of motility.

3. Motility is not dependent upon the presence of oxygen, but added substrate in the form of utilizable sugar is essential for prolonged activity.

4. Prolonged exposure to air or to oxygen may result in failure of motility and of glycolysis.

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THE ABSORPTION OF CAROTENE FROM ISOLATED INTESTINAL LOOPS[1]

J. LOGAN IRVIN, JOSEPH KOPALA AND CHARLES G. JOHNSTON

From the Department of Surgical Research, Wayne University College of Medicine, Detroit

Recently it has been shown that disturbances in the absorption of fat-soluble vitamins or their precursors can exist in humans suffering from biliary insufficiency and can lead to serious clinical manifestations of avitaminosis in spite of an adequate supply of these vitamins in the diet (2, 3, 5, 18). In view of the clinical importance of the intestinal absorption of fat-soluble vitamins and of the incomplete knowledge regarding the rôle of bile in this process, further study appeared to be desirable. The ease and accuracy with which carotene may be determined recommended it for this study.

Greaves and Schmidt (13, 14) were the first to demonstrate that bile acids are necessary for the absorption of carotene by rats. Their criterion for the absorption of carotene was the restoration of a normal vaginal smear picture in vitamin A deficient rats. Therefore, their results were only qualitative or roughly quantitative. Other investigators have demonstrated that a certain amount of normal absorption of fat is necessary for transportation of carotene across the intestinal wall. On a fat-free diet, rats assimilate only a small fraction of the amount that is completely utilized when 10 per cent fat is included in the diet (1). Clausen (6) has studied rates of absorption of carotene by determination of this provitamin in blood plasma.

In the present study the absorption of carotene has been investigated by the use of dogs with isolated intestinal loops. Known quantities of carotene were placed in the loops under a variety of conditions and the amount of absorption was determined by carotene analyses of the contents of the intestinal loop remaining after a period of absorption. This technique is advantageous for a number of reasons. It permits direct com-

parison of the absorption in a large series of experiments on the same animal under a variety of conditions thus allowing a more accurate statistical analysis of the results. The use of such loops eliminates errors due to variations in the effective absorbing surface which would be unavoidable in the intact animal. In addition, determinations of carotene remaining in the loops after absorption periods would seem to be a more accurate means of estimating the amount of absorption than biological tests or than plasma analyses for carotene. Analysis of plasma for carotenoids provides a measure of the difference between the rate of absorption of carotene from the intestine and the rate of its absorption and utilization by the tissues and other body fluids rather than a direct index of intestinal absorption alone. Such plasma carotene determinations are valuable but do not provide a means for the quantitative evaluation of the effectiveness of various substances on the absorption of this provitamin. The use of intestinal loops obviates such difficulties.

EXPERIMENTAL. *Analytical method*. For the determination of carotene in the stock solutions and in the contents of the intestinal loops, the solution is saponified with 20 cc. of a freshly prepared solution of KOH in alcohol for each gram of sample. The saponification flasks are fitted with reflux condensers, and the contents are boiled on a steam bath for 30 minutes, or until saponification is complete. A stream of nitrogen is bubbled through the solution during the saponification period to prevent oxidation of carotene. The contents of the flask are then cooled, 50 to 100 cc. of ethyl ether are added, and after shaking for a minute or so and allowing to settle, the ether-alcohol mixture is decanted into a separatory funnel. This is repeated two additional times with small amounts of ether; then the residue in the flask, which sometimes forms an adherent mass, is broken up by shaking with a small quantity of 95 per cent ethyl alcohol. After two or three additional extractions with ether, the ether extract usually comes off colorless, and the residue is then discarded. About 250 cc. of ether are usually required for complete extraction. Next about 100 cc. of distilled water are poured gently through the alcohol-ether solution in the separatory funnel. The alkaline alcoholic-aqueous solution which separates is withdrawn from the bottom of the funnel and is re-extracted by shaking with ethyl ether in another separatory funnel. If an emulsion is encountered, it may be cleared by adding a little ethyl alcohol. The ether extracts are combined and washed with distilled water until free from alkali. Washing three or four times by pouring the water through the ether solution and down the sides of the funnel removes most of the alkali. The remainder is removed by gently shaking the ether solution with small quantities of water until the wash water no longer gives a color with phenolphthalein.

The ether solution containing carotene is dehydrated by permitting it to stand over anhydrous sodium sulfate for several hours. Then the ether

solution is decanted and the sodium sulfate is washed several times with anhydrous ether to remove any adsorbed carotene. The combined extracts are evaporated under a stream of nitrogen in an all-glass distillation apparatus. The final stage of evaporation to complete dryness is carried out under reduced pressure. The carotene residue in the flask is dissolved in petroleum ether (30–60° boiling fraction), the solution is made up to an appropriate volume in a volumetric flask, and readings are made in an Evelyn photoelectric colorimeter (11) using filter number 440 (transmission limits 410 to 475 mμ. Mean transmission at 440 mμ). Carotene is estimated from these galvanometer readings by reference to a standard calibration curve obtained by reading known dilutions of a sample of pure crystalline β-carotene. This standardization curve is shown in figure 1.
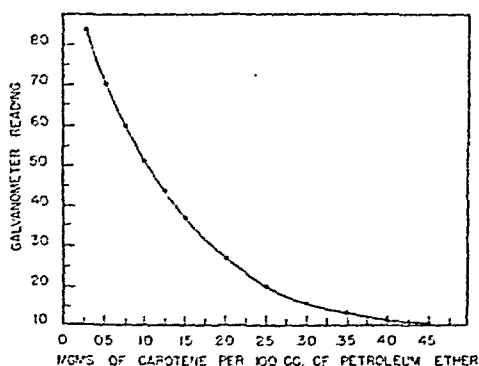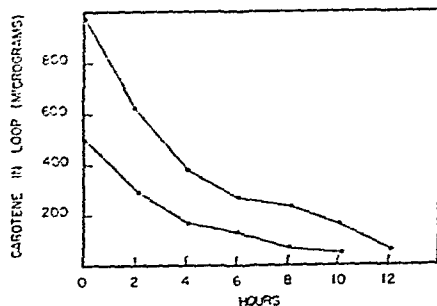
Fig. 1   Fig. 2

Fig. 1. Standardization curve of β-carotene in petroleum ether. Percentage transmission at 440 mμ.

Fig. 2. Carotene absorption curves. Effect of carotene concentration on the rate of absorption in the presence of bile and pancreatic lipase.

Kimble (17) has used a photoelectric colorimeter for the determination of carotene in plasma. According to Kimble's procedure, the carotene extracted from the plasma is dissolved in petroleum ether for the photoelectric colorimeter readings. She has found that the color of carotene dissolved in petroleum ether does not follow Beer's law perfectly. Our results verify that conclusion. However, the L values conform fairly well in the middle region of the curve, and the determinations are quite satisfactory when dilutions are made so that the galvanometer readings fall along this portion of the standardization curve.

The results of analyses of standard stock solutions of carotene in cottonseed oil are presented in table 1. It may be seen in line one, column five, that the analyses yield an average of 99.1 per cent theoretical with a probable error of $\pm$ 2.1 per cent. Analyses of mixtures of known quantities of carotene in oil with lipase and bile are presented (line three). The

accuracy is of the same order. The accuracy also is not affected by digestion of these mixtures for six hours at 37°C. (line four).

Preparation of loops and technique of experiment. The loops were prepared by the technique described by Johnston (16). Only loops of the upper jejunum were used. Carotene was introduced into the loops in the form of saturated solutions of crystalline β-carotene in cotton-seed oil. Johnston's (16) double balloon technique was used for closing the loops, and the solutions were injected by means of the central catheter tube. After the introduction of the oil solutions, the tube was washed down with two 5 cc. portions of distilled water. A check on the accuracy of the entire technique was obtained by a number of experiments on the recovery of carotene placed in the intestinal loop and then withdrawn immediately

TABLE 1

Accuracy of method for determination of carotene in intestinal contents

TYPE OF ANALYSIS	CAROTENE THEORETICAL	NUMBER OF DETERMINATIONS	CAROTENE DETERMINED (AVERAGE)	CAROTENE RECOVERED
	micrograms		*micrograms*	*per cent*
Carotene in oil (1 cc. samples).......	490	14	486	99.1 ±2.1
Carotene in oil (5 cc. samples).......	2450	17	2421	98.9 ±2.3
Carotene solution (1 cc.) + 1 gram bile + 1 gram lipase...................	490	11	483	98.5 ±2.6
Carotene solution (1 cc.) + 1 gram bile + 1 gram lipase + 10 cc. H$_2$O (6 hours at 37°C.)...................	490	9	481	98.1 ±2.9
Immediate recovery of carotene from loop in presence of bile and lipase....	490	12	466	95.3 ±3.4
Immediate recovery of carotene from loop in presence of bile and lipase..	2450	7	2355	96.1 ±3.2

without a period of absorption. In these experiments, mixtures of known amounts of carotene in oil with bile and lipase were placed in the loops as described, and the loop contents were withdrawn immediately by suction on the rubber tube with a syringe. The loop was washed out three times with emulsions of 5 cc. of oil in distilled water. The loop contents and washings were combined and analysed in the usual manner. The averages shown in table 1, column five, lines five and six are satisfactory.

Effect of various substances on the absorption of carotene. In the absorption experiments a similar procedure was followed. Stock standard solutions of carotene in oil were placed in the loop along with the materials whose effects on the absorption were to be studied. Each solution was neutralized to pH 7 before introduction into the loop. After the absorption period, the loop contents were aspirated with a syringe, and the loop was

washed out several times with emulsions of oil and water as described above. The combined loop contents and washings were analysed and the

TABLE 2

Effect of various substances on the absorption of carotene from an intestinal loop

(Length of loop—8 inches; all absorption periods were 4 hours)

ABSORPTION CONDITIONS	NUMBER OF EXPERI-MENTS	AMOUNT OF CAROTENE IN LOOP		CAROTENE ABSORBED (AVERAGE)	CAROTENE ABSORBED (PER CENT OF INITIAL)
		Initial	Final (average)		
		micrograms	*micrograms*	*micrograms*	
Carotene in 1 cc. of oil + 10 cc. of water...................	13	473	461	12	2.5 ±2.4
Carotene in 5 cc. of oil + 10 cc. of water...................	8	473	457	16	3.4 ±3.1
Carotene in 1 cc. of oil + 1.2 grams of dried hog bile + 10 cc. of water.................	20	473	398	75	15.9 ±3.8
Carotene in 1 cc. of oil + 1 gram of dried pancreatic lipase + 10 cc. of water..............	19	473	428	45	9.5 ±3.6
Carotene in 1 cc. of oil + 1.2 grams of dried hog bile + 1 gram of dried lipase + 10 cc. of water....................	23	473	262	211	44.6 ±6.3
Carotene in 1 cc. of oil + 1 gram of dried ox bile + 1 gram of dried lipase + 10 cc. of water.	20	473	229	244	51.6 ±5.6
Carotene in 1 cc. of oil + 0.74 gram of sodium glycocholate* + 1 gram of dried lipase + 10 cc. of water..............	15	473	309	164	34.6 ±4.9
Carotene in 1 cc. of oil + 0.65 gram of sodium cholate + 1 gram of dried lipase + 10 cc. of water.................	15	473	294	179	37.8 ±4.1
Carotene in 1 cc. of oil + 0.63 gram of sodium desoxycho-late† + 1 gram of dried lipase + 10 cc. of water...........	15	473	162	311	65.7 ±6.3
Carotene in 1 cc. of oil + 1.2 grams of dried hog bile + 1 gram of dried lipase + 5 cc. mineral oil + 10 cc. of water..	5	473	425	48	10.1 ±5.5

* Synthesized by the method of Cortese and Bauman (7).

† Courtesy of Riedel-de Haen, Inc.

amount of carotene absorbed was determined by the difference between the amount of carotene placed in the loop initially and the amount remaining after the absorption period. The results are recorded in table 2.

All results presented in table 2 were obtained with the same loop dog in order that direct comparisons may be made between the different absorption conditions. However, similar experiments have been performed with three other dogs with comparable results.

Effect of carotene concentration on its absorption. In order to determine the effect of the initial concentration of carotene on the rate of absorption of this provitamin from the intestine, numerous absorption experiments were conducted for various absorption intervals with two different initial amounts of carotene (500 and 975 micrograms) in cotton-seed oil. In each experiment 1 gram of dried lipase[2] and 1 gram of desiccated ox gall-bladder bile[3] were added, and the mixture was washed into the loop with 10 cc. of distilled water. The results plotted in figure 2 were obtained with the same dog for comparison. All points shown in this graph represent the average of ten different experiments. The rate of absorption is somewhat greater when the larger initial concentrations of carotene are employed.

DISCUSSION. It may be seen from table 2 that the absorption of carotene from the loop in the presence of oil but in the absence of bile and lipase is very slight if, indeed, it is significant at all. When lipase is added, a small but statistically significant amount of carotene is absorbed. Similarly, significant absorption occurs in the presence of hog bile alone. However, when both one gram of dried hog bile and one gram of desiccated lipase are placed in the loop with the carotene solution, much larger amounts of carotene are absorbed during a period of the same duration. The effect of bile on the absorption of fat-soluble vitamins has received much attention, but the beneficial effect of pancreatic lipase on such absorption has not been emphasized. However, recently May et al. (18) have indicated that absorption of carotene is defective in children with pancreatic disease or obstruction of the pancreatic duct as well as in those with biliary obstruction. Our results would suggest that the defective absorption in such cases may be due to deficiency of pancreatic lipase. The decrease in the absorption of carotene in the absence of pancreatic lipase may be explained as due to disturbed fat digestion and absorption which would lead to retention of the fat-soluble carotene in the intestine. In this regard, the effect of mineral oil on the absorption of carotene may be commented upon. Table 2 indicates that mineral oil placed in the intestinal loops decreases the absorption of carotene even in the presence of bile and lipase. Such an effect has been reported by others (4, 9). This also may be explained on the basis of the solubility of carotene in this oil. Since only traces of mineral oil are absorbed, the oil-soluble carotene will be retained in the intestine.

Hog and ox gall-bladder bile are almost equally effective in promoting

[2] Difco Laboratories.

[3] Desiccated hog and ox gall-bladder bile were generously donated by Parke, Davis and Co.

absorption of carotene. In table 2 it would appear that ox bile is some-what more effective than an equivalent quantity of hog bile, but this difference has little statistical significance. The amounts of desiccated ox and hog gall-bladder bile used in these experiments were equivalent (on a mol basis) in their content of total bile salts. The effectiveness of hog bile is interesting in view of the fact that the bile acids of hog bile are quite different qualitatively from those found in dog bile (19). On the other hand, the bile acids of dog bile and ox bile are quite similar, the only important difference being that ox bile contains bile acids conjugated with glycine as well as with taurine (19).

The results of experiments on the effect of pure bile salts on the absorp-tion of carotene are interesting from the standpoint of the theories con-cerning the mechanism of absorption. In these experiments (recorded in table 2) the amounts of bile salts used were equivalent on a mol basis to the total bile salt content of the gall-bladder bile used in the previous experiments. It may be seen that the unconjugated bile salts are more effective than the conjugated salt. The positive results with sodium glycocholate are contradictory to the experiments of Greaves and Schmidt (15), but the conditions are not entirely comparable since they used rats and their criterion for absorption was the restoration of a normal vaginal smear picture. Of the unconjugated bile salts, sodium desoxycholate has greater effect than sodium cholate. These results may be interpreted in relation to the theory of Wieland and Sorge (23), Fürth and Minibeck (12) and Verzár (21, 22) which explains the effect of bile salts on the absorption of fat and fat-soluble materials as being due to the formation of choleate complexes with the bile salts. It has been pointed out (20) that stable, crystallizable choleate complexes are formed chiefly by desoxycholic acid and its salts. Von Euler and Klussmann (10) have described water soluble, diffusible complexes of the bile salts with carotene. Conjugation destroys the ability of desoxycholate to form stable, crystallizable choleates (8). However, in spite of their inability to form crystalline choleate complexes, conjugated bile salts such as sodium glycocholate are effective in promoting absorption of carotene (table 2). It should be pointed out also that bile salts in bile are principally in the conjugated form (19), but bile never-theless is quite effective in aiding the absorption of carotene. Therefore, the importance of choleate formation in the rôle of absorption of this fat-soluble vitamin should be minimized although the concept cannot be abandoned completely since Verzár and Kuthy (21) have presented evidence that conjugated bile acids can form complex choleates in solution even though the complexes cannot be isolated and crystallized. The marked surface activity of bile salts which permits emulsification of fat and dispersion of the fat soluble vitamins probably plays an important rôle in the absorption of carotene, but this may not be the complete ex-planation of the mechanism.

SUMMARY

The absorption of carotene from isolated intestinal loops of dogs has been studied by means of photoelectric colorimetric determinations of the provitamin in the loop contents before and after absorption periods. Carotene is not absorbed in an analytically significant amount when placed in the loops in concentrated solutions in cotton-seed oil without bile and pancreatic lipase. When carotene solutions are given along with hog or ox gall-bladder bile, significant amounts are absorbed. Similarly, small amounts are absorbed when carotene in oil is introduced into the loops with pancreatic lipase. However, when the carotene solution is placed in the intestinal loops with *both* bile and pancreatic lipase, much larger amounts are absorbed.

Pure bile salts are capable of promoting the absorption of carotene. Sodium desoxycholate is the most effective bile salt of those studied, but sodium cholate and sodium glycocholate are also effective. These results and other evidence in the literature suggest that the choleate theory of the mechanism for the absorption of carotene should be minimized but not abandoned completely.

In the presence of identical amounts of bile and lipase, the rate of absorption of carotene is greater when the initial amount of carotene in the loop is increased. Reports on the inhibiting effect of mineral oil on the absorption of carotene are confirmed.

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THE EFFECT OF THIAMIN HYDROCHLORIDE ON THE MUSCULAR DYSTROPHY OF AVITAMINOSIS-E

ARTHUR D. HOLMES AND MADELEINE G. PIGOTT

From the Research Laboratories, The E. L. Patch Company, Boston, Mass.

Several years ago Evans and Burr (1928) described a paralysis which developed in suckling rats that were fed a vitamin E deficient ration. They concluded that the paralysis was a true deficiency disease which could be produced or prevented by regulating the amount of vitamin E consumed by the young rats. Olcott and Mattill (1934) observed paralysis in 30 to 40 young mother rats which had been fed a vitamin E free diet for about four weeks. Ringsted (1935) reported paresis in adult rats that had received an inadequate supply of vitamin E for four to seven months. Blumberg (1935) also noted muscular disturbance and emaciation in female rats fed a vitamin E deficient ration for forty to fifty weeks. Morgulis and Spencer (1936) were able to prevent or cure muscular dystrophy in rabbits by supplementing the experimental ration with materials containing vitamin E but they concluded that paralysis and muscular dystrophy are not due to the same cause. Burr, Brown and Moseley (1937) state that vitamin E paralysis is easily induced in suckling young rats, that old age paralysis may also be due to vitamin E deficiency and that the paralysis is neither completely motor nor completely sensory. Morgulis, Wilder and Eppstein (1938) concluded that of the essential nutritional factors necessary for the integrity of the skeletal muscles the fat-soluble one is very closely associated or perhaps identical with vitamin E. This survey of the literature indicates that these and other investigators are in agreement that the various forms of muscular dystrophy occur in animals receiving vitamin E deficient rations.

Olcott (1938) commented upon the sudden occurrence of paralysis in second generation rats and stated that the paralysis in the young of vitamin E deficient female rats is due to lesions of the skeletal muscle similar to those in so-called nutritional muscular dystrophy of herbivora and he apparently considers this condition due largely if not wholly to the lack of vitamin E.

During the course of conducting vitamin E assays in this laboratory three female rats which were being maintained on ration I, a modified Olcott and Mattill (1934) ration, consisting of casein 20 per cent, sucrose

44 per cent, lard 22 per cent, yeast 8 per cent, salt mixture 4 per cent, and cod liver oil 2 per cent, produced litters of living young. At approximately weaning age the young rats developed various degrees of muscular dystrophy and paralysis. They were immediately weaned but continued to receive the same vitamin E deficient ration. When the young rats no longer received any of their mothers' milk and were entirely dependent upon ration I they began to improve. After a short time they appeared normal and grew to maturity. In seeking a possible explanation for this situation, attention was directed to vitamin B_1 and some experiments were conducted to determine the effect of administering thiamin hydrochloride to weanling rats afflicted with muscular dystrophy and paralysis.

Litter 4, of ten rats, was born to a female with avitaminosis-E. This animal had been fed ration II, a modification of Mason and Bryan (1938) diet 68. Its approximate composition was: casein (commercial) 20.0 per cent, corn starch 49.1 per cent, lard 18.2 per cent, Osborne and Mendel (1913) salt mixture 3.6 per cent, brewer's yeast 7.3 per cent and cod liver oil 1.8 per cent. Five of the young were raised and when they were twenty days old two died during the night. Previously they did not show any symptoms of muscular dystrophy, received no medication and the exact cause of their death was unknown. Two others developed pronounced but not severe muscular dystrophy. These animals were sufficiently active to be able to eat their vitamin E deficient ration in the usual manner. Two doses of thiamin hydrochloride, of six gamma each, were administered by mouth. Definite improvement was shortly evident and apparently complete recovery was obtained within a few days. The fifth young rat developed severe muscular dystrophy on the twentieth day of life. It refused all food and was fed, with a medicine dropper, the vitamin E-deficient ration II, mixed with distilled water. This was supplemented with a solution of vitamin B_1 (thiamin hydrochloride), six gamma, administered by mouth. Definite improvement and increased appetite were noted within twenty-four hours. However, it was still necessary to feed ration II mixed with water by a dropper and the rat still dragged its hind legs. The following day a second dose of twelve gamma of vitamin B_1 was administered. The rat made decided improvement and ate of its own accord. Two days later it ate and walked normally and was apparently cured without further treatment.

Litter 5 was born to a female which had received ration II continuously since she was fourteen days old. The five young rats developed pronounced muscular dystrophy at twenty-two days of age. A dose of six gamma of thiamin hydrochloride was administered to each animal during the morning. In the late afternoon each animal received an additional twelve gamma of thiamin hydrochloride. One rat developed an enlarged head (edematous) which also improved following the administration of

synthetic vitamin B_1. In two days these rats were cured with the exception of a slight flexure of the toes which later corrected itself without further dosage. In all instances the young rats were kept on the same vitamin E low diet as their mother except in the case where forced feeding was necessary for existence, then the diet was mixed with distilled water and fed by a medicine dropper.

Litter 6 was produced by a female that had been fed ration II from the fourteenth day of life. The entire litter of five rats was raised to twenty days of age. At that time two of the animals died suddenly during the night without having exhibited definite symptoms of muscular dystrophy. Previous to their death these rats apparently were definitely uncomfortable although they were very active. Three of the litter of five which were raised to weaning developed severe muscular dystrophy at twenty days of age. During the twentieth night one of the young rats died without having received any medication. The two remaining rats developed very severe muscular dystrophy. They became badly emaciated and refused to eat. A dose of fifteen gamma of vitamin B_1 thiamin hydrochloride, in solution, was administered by mouth. Due to difficulty in administration approximately only one-half of this amount was consumed. Little or no change in the animals' condition was noted except that there was a slight increase in an effort to take food from a dropper (vitamin E deficient ration II mixed with distilled water). Previous to the administration of vitamin B_1, the rats exhibited an extremely helpless and dazed condition (as in infancy). Two days later twelve gamma were again given by mouth to each rat. Late in the afternoon each animal was given a second dose of twelve gamma of thiamin hydrochloride. A decided improvement followed. The animals showed definite interest in food although refusing to eat of their own accord. The next day twelve gamma of thiamin hydrochloride were administered to each rat. There was a further slight improvement in physical condition but the appetite was decidedly improved. Five days from the onset the rats were apparently free of the muscular dystrophy except a slight flexure of the toes remained. The sixth day after medication was started the animals were free from the flexure of the toes and seemed to be completely cured. A total of approximately forty-three gamma or fourteen International Units of thiamin hydrochloride was administered to each animal during a period of four days.

The amount of thiamin hydrochloride which was required to produce recovery in the afflicted rats varied from twelve to forty-three gamma or from four to fourteen International Units of vitamin B_1. Apparently the more severe the condition of the rat, the larger the amount of thiamin hydrochloride required.

A survey of the literature did not reveal any instance in which this

amount of thiamin hydrochloride had been administered within four days to weanling rats of about forty grams weight suffering with muscular dystrophy following the continuous feeding of their mother with a vitamin E-deficient ration.   However, the results obtained in this study indicate that the recoveries which followed the administration of vitamin B_1 to young rats with muscular dystrophy and paralysis resulted from the massive doses administered.

As further evidence that the administration of massive doses of thiamin hydrochloride caused a permanent cure of the weanling rats afflicted with muscular dystrophy and paralysis, all the rats grew to maturity and have remained in good health during six months of adult life.

SUMMARY

Studies upon young weanling rats which had developed various stages of muscular dystrophy, due to a vitamin E-deficient diet, revealed that there was a definite response to the oral administration of massive doses of vitamin B_1 (thiamin hydrochloride).

The response of the individual rat to a definite dose varied according to the severity of muscular dystrophy.

The total amount of thiamin hydrochloride necessary to effect a cure ranged from 4 to 14 International Units per rat.

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THE PENETRATION OF RADIOACTIVE SODIUM AND PHOSPHORUS INTO THE EXTRA- AND INTRA-CELLULAR PHASES OF TISSUES[1]

JEANNE F. MANERY AND WILLIAM F. BALE

From the Departments of Physiology and Radiology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

Sodium has received considerably less attention in the study of tissue electrolytes than chloride, undoubtedly because of the difficulty of sodium micro-chemical methods. It has been generally assumed to be in the extracellular phase because of the evidence that most of the chloride in muscle is extracellular (7) and because of the fact that in some tissues the sodium:chloride ratios are the same as in plasma. The tendency to extend to all tissues, concepts which may aptly describe muscle, was criticized by Amberson et al. (1) and by Manery and Hastings (18), the latter showing that the sodium:chloride ratio differed appreciably in many tissues from that in plasma. An "excess" chloride was reported (18) in rabbits in blood, connective tissues, testes, gastric mucosa and in the liver of rats (13a, and this paper), and an "excess" sodium in cartilage, spinal cord and intestinal wall (18) which suggests the existence of both intracellular sodium and chloride. It is of some interest, too, that in certain muscles a small "excess" sodium has been reported but this is not true for all muscle tissue. It was located in frog (6) and in dog skeletal muscle (11, 12) but not in the skeletal muscles of the rabbit (11, 18). To conform to the simple morphological division into intra- and extracellular phases, this excess sodium has been allocated to the intracellular phase. In view of these findings and of the fact that the red cells of many species (15) contain sodium instead of potassium, it seems possible for sodium to function as an intracellular ion. This report shows how radioactive isotopes were used to investigate such a possibility.

The general scheme of dividing the tissues into two phases, intra- and extracellular, has been adopted. The limitations of this simple procedure are fully realized but its lack of complexity makes it useful. The following studies have been carried out and are reported herein:

a. The rate and extent of the penetration of radioactive sodium into a

[1] The data reported here were presented at the meeting of the American Physiological Society, Toronto 1939 (see (16)).

variety of mammalian tissues with a view to determining the proportion of sodium already present as intra- or extracellular; b, a comparison of the behaviour of radioactive phosphorus, phosphate being typically an intracellular ion, with that of sodium which is essentially extracellular; c, the penetration of both isotopes into red cells; d, the use of radioactive sodium for the determination of the extracellular fluid of the organism as a whole.

METHODS AND CALCULATIONS. Sodium chloride and red phosphorus were bombarded[2] with deuterons to obtain radioactive sodium ($_{11}Na^{24}$) of half-life 14.8 hours, and radioactive phosphorus ($_{15}P^{32}$) of half-life 14.5 hours, respectively. Before the sodium chloride was used, sufficient time elapsed for the complete dissipation of the radioactivity due to the chlorine isotope which is produced simultaneously.

The sodium isotope was given to animals in 0.8 to 1.0 per cent solutions, 2.5 to 3.0 ml. per 100 grams of body weight being injected intraperitoneally into rats and $\frac{1}{10}$ of this dose into the ear veins of rabbits. The solutions contained not more than 2.0 millicuries of radioactivity per liter. The radioactive phosphorus was injected as a trace of sodium dihydrogen phosphate dissolved in an isotonic sodium chloride solution, the quantity of solution being the same as in the case of radioactive sodium. The animals were killed by decapitation at various periods of time after injection. They were bled and the tissues, plasma, peritoneal fluid and urine sampled and prepared for analysis. Tissues were freed from large blood vessels, connective tissue capsules and fascia where possible. In the kidney an attempt was made to exclude the medulla and use only cortex. Chemical determinations of chloride and sodium were made according to the procedures and with the accuracy previously described (17, 18).

The radioactivity of each sample was determined by means of a Geiger-Müller counter employing the counting tube and technique described by Bale et al. (2). Because this apparatus was designed to determine the activity in 2 ml. of solution, two methods of ashing the tissues were utilized. In some experiments they were dissolved in nitric acid on a steam bath. Weighing bottles were used and the solution diluted to the desired volume and weighed. Since a 2 ml. aliquot was taken for analysis the specific gravity of the solution had to be known. Although this is an expedient method it is more hazardous and hence less desirable than the second procedure adopted, namely, ashing in a muffle furnace overnight at about 530°C. The latter method is particularly convenient because the ash can be dissolved in 1 ml. of N HCl and diluted with water at the time of counting to any volume necessary to bring the number of counts per minute within the range for which the instrument has been calibrated.

All solutions were counted for 3 to 5 periods of 5 minutes and each figure reported is the average of the counts for several periods. To calculate the results a distilled water background count was first subtracted from each unknown which was then calculated to an arbitrarily chosen but constant potassium acetate count. With an isotope of short half-life a standard radioactivity decay curve was employed to take account of the decay which occurred during the course of the experiment.

The accuracy obtained with this method may be expressed as the per cent difference between the counts given by duplicate tissue samples, which is 7 per cent (average of 20 pairs).

The counts alone for each tissue are quite meaningless, but their relative values are informative when related to some constant such as the number of counts injected or the plasma count. The plasma is a better point of reference than whole blood because of the possible accumulation of the isotope in the blood cells. We have calculated the ratio of the tissue concentration to the plasma concentration of a, radioactive sodium (Na^{24}); b, chemically determined sodium (Na), and c, chemically determined chlorine (Cl). In any tissues where all of the sodium and chloride are extracellular the sodium and chloride will be contained in the interstitial fluid which is undoubtedly a plasma ultrafiltrate. Hence when proper corrections have been applied to the above ratios for the water content of plasma and the Gibbs-Donnan equilibrium (see below), the resulting values will measure the volume of the extracellular water. In keeping with symbols previously used, this calculated value has been designated $(H_2O)_E$. Such a calculation obviates any assumption with regard to the solid content of the extracellular phase (see Manery and Hastings, 18). It is not our belief that sodium and chloride are entirely extracellular in all tissues of the body (5, 17, 18). However, the analytical figures for each tissue were subjected to the same method of calculation, for convenience in comparing the tissue : plasma ratios.

The method of calculation follows:

$$(H_2O)_E\,Cl = \frac{\text{tissue Cl (m.eq. per kgm.)}}{\text{plasma Cl (m.eq. per liter)}} \times 0.95 \times 0.93 \times 100 \ . . (1)$$

$$(H_2O)_E\,Na = \frac{\text{tissue Na (m.eq. per kgm.)}}{\text{plasma Na (m.eq. per liter)}} \times \frac{0.93}{0.95} \times 100 \ . . . . . (2)$$

$$(H_2O)_E\,Na^{24} = \frac{\text{tissue counts per min. per kgm.}}{\text{plasma counts per min. per liter}} \times \frac{0.93}{0.95} \times 100 \ . . . (3)$$

$$(H_2O)_E\,P^{32} = \frac{\text{tissue counts per min. per kgm.}}{\text{plasma counts per min. per liter}} \times 0.95 \times 0.93 \times 100 \ . . (4)$$

Where $(H_2O)_E$ is expressed in grams per 100 grams of fresh tissue, 0.95 is the Gibbs-Donnan ratio and 0.93 the correction for the water content of plasma. These values may be referred to in the text as Cl, Na, Na^{24} and P^{32} ratios respectively.

In addition calculations were made of the proportion of the body into which the absorbed radioactive sodium was distributed. If sodium is entirely extracellular this value will represent the volume of the extra-cellular water in the body as a whole.

Per cent distribution (gram per 100 grams body wt.) =

$$\frac{\text{Counts injected} - \text{counts excreted}}{\text{plasma counts per liter}} \times \frac{930}{0.95} \times \frac{100}{\text{body wt. (gram)}} \cdots (5)$$

The amount of the dose injected which is found in the plasma at the time of decapitation is of some significance. Using Skelton's values (19) for the blood contents of rats and rabbits which are 5.5 and 6.75 per cent of the body weight respectively, the calculation is as follows:

Per cent dose in plasma =

$$\frac{\text{per cent pl.} \times \text{per cent bl.} \times \text{body wt. (gram)} \times \text{pl. count per ml.}}{\text{counts injected} \times 100} \cdots (6)$$

Where per cent pl. = plasma volume in per cent of whole blood.

and per cent bl. = blood content in per cent of body weight.

RESULTS. 1. *Na^{24} distribution and the extracellular phase.* Because many tissues were analysed in each of the 18 animals studied it will be impossible to present all results in detail. The actual experimental figures are given in the case of only one animal (table 1) but sufficient data are presented in the other tables to permit calculation of any desired analytical values.

a. *Na^{24} distribution at equilibrium.* The first experiment (table 1) was designed to ascertain the extent to which tissues were penetrated by injected radioactive sodium, in a period of time adequate for equilibrium to be established. To decrease the sampling error both chemical sodium and radioactivity were determined on the same sample. In columns (1) and (2) are listed the chloride and sodium concentrations of tissues. These compare well with those previously determined (18) on normal rabbits receiving no injections, showing that neither the solution injected nor the radioactivity of the sample appreciably altered the normal electrolyte concentrations. The values for $(H_2O)_E$ in liver, brain, perirenal fat and all muscles are the same whether calculated from chloride or sodium. In tendon, kidney, testes, spleen, parts of the gastrointestinal tract and bladder the chloride ratios exceed the sodium values. In cartilage and sciatic nerve the reverse is true. These results, although uncorrected for blood and fat, in general confirm earlier findings. It has already been suggested

TABLE 1

Analyses and calculations pertaining to rabbits injected intravenously with radio-active sodium

FRESH TISSUE	RABBIT 1 (1 HR. 8 MIN.)							RABBIT 2 (13 MIN.)
	m.eq. per kgm. or l. of		Counts per min. per m.eq. ÷ 10³	Per cent dose of Na²⁴ in whole organ	$(H_2O)_E$ in grams per 100 grams calculated from			
	(1) Cl	(2) Na	(3) S.R.	(4)	(5) Cl	(6) Na	(7) Na²⁴	(8) Na²⁴
Skin.................	67.8			11.6	62.5			42
Tendon..............	80.9	74.6	13.0		74.6	52.1	53	39
Kidney*.............	55.7	65.6	11.8	1.2	51.3	45.8	42	33
Testes*.............	52.6	40.7	12.5		48.5	28.4	28	18
Ear cartilage.........	60.3	138.6	13.9		55.6	96.6	104	71
Spleen..............	46.6	43.2	11.8	0.1	43.0	30.1	27	23
Liver*..............	24.2	32.5	12.7	4.7	22.3	22.6	22	17
Pyloric mucosa.......	72.8	47.5	14.2		67.1	33.1	36	33
Pyloric muscle.......	47.0	36.9	14.3		43.3	25.7	29	23
Fundic mucosa.......	119.6	25.2	12.2		110.1	17.6	17	
Fundic muscle.......	72.6	42.0	12.1		67.0	29.3	27	
Small intestine.......	40.7	47.7	12.2	5.1	37.5	33.2	32	
Sciatic nerve.........	49.5	77.7†	6.6	0.2	45.7	*54.2*	*28*	
Cerebrum............	39.9	51.5	3.8	0.3	36.8	*35.9*	*11*	4.3
Bladder.............	62.6	62.9	11.7		57.8	43.8	40	49
Heart...............	32.0	42.6	13.2	1.1	29.5	29.7	30	28
Abdominal muscle...	23.6	25.4	13.9	31.0	21.8	17.7	19	11
Gastrocnemius.......	13.1	14.4	13.1		12.1	10.0	10	7.7
Perirenal fat*........	7.9	11.0			7.3	7.7		
Urine*..............	13.1	Negl.						
Plasma*.............	95.8	140.1	12.9					
Whole blood*........	85.4	98.7	12.0	17.6				
Rest of body........				2.1				

Per cent distribution (grams per 100 grams body weight), for rabbit 1 = 25, for rabbit 2 = 21.

Per cent of injected dose in plasma, for rabbit 1 = 14, for rabbit 2 = 20.

Negl. = negligible.

Rabbit 1 was killed in 1 hour 8 minutes after intravenous injection, rabbit 2 in 13 minutes. Tissues of rabbit 1 were ashed in a muffle furnace for Na and Na²⁴ determinations, those of rabbit 2 ashed in nitric acid on a steam bath.

Liter units apply to urine, plasma and whole blood; kilogram units to all other tissues.

* Figures pertaining to these tissues are averages of duplicates. All others represent single analyses.

† From Manery and Hastings (18).

(18) that the sodium figure gives a more reasonable value for the extra-cellular phase than the chloride figure in a tissue in which the chloride ratio exceeds the sodium ratio.

If the sodium is entirely extracellular it must be in ionic equilibrium with plasma and any injected radioactive sodium should freely exchange with sodium already present in the tissues. In this case the volume of the extracellular water based on Na^{24}, i.e., $(H_2O)_E Na^{24}$, should be equal to $(H_2O)_E Na$. If, on the other hand, some of the sodium is contained within cells which are impermeable to it, $(H_2O)_E Na$ will exceed $(H_2O)_E Na^{24}$ by the amount of non-diffusible intracellular sodium present. By comparing the values of extracellular water in column (6) with the corresponding figure in column (7) a striking agreement is demonstrated in every tissue studied except brain and sciatic nerve. The ratios are almost identical in many tissues and even the greatest difference does not exceed 10 per cent, whereas in brain and nerve the Na^{24} ratio only attains 30 and 50 per cent respectively, of the magnitude of the Na ratio. There is no correspondence whatsoever between the quantity of extracellular water calculated from Cl and that from Na^{24}, even in nerve and cartilage where the sodium concentration exceeds the chloride. Furthermore, there is no evidence of a tendency for the Na^{24} ratio to exceed that of chemical sodium showing that the radioactive isotope does not concentrate in any tissue. The experiment demonstrates that, with the exception of nervous tissue, in 1 hr. 8 min. injected radioactive sodium becomes distributed between tissues and plasma in precisely the same ratio as normally occurring sodium.

In column (3) we have listed calculations of the counts per milliequivalent of sodium ÷ 10^3 (S.R. = specific radioactivity) found in each tissue. If tissue sodium is in free diffusion equilibrium with plasma sodium then Na^{24} should mix with the tissue sodium in the same proportion as it does with the plasma sodium. The value of S.R for plasma is 12.9. The table shows that for all tissues except nervous tissues S.R. very closely approximates the plasma value. Actually, excluding cerebrum and nerve, the average is 12.8. The close correspondence of tissue S.R. values to the plasma value merely illustrates in another way the completeness with which Na^{24} has become mixed in an hour with the sodium of the body.

Bladder urine contained very little chloride and insufficient sodium to be analyzed by this chemical method. The radioactivity was also negligibly small at the time at which other tissues were counted. Hence the data obtained on this animal are so nearly complete that we have attempted to account for the dose injected by estimating the amount found in each organ. Skelton's tabulation (19) of the percentage weight of rabbit organs in relation to body weight was used and also the Na^{24} concentration found for skin in rabbit 3. The results are listed in column (4) in per cent of the dose injected. Muscle, whole blood and skin contain much the largest proportion of the injected sodium. The sum for all organs is 75 per cent but we have omitted the skeleton which makes up 12.4 per cent of the body weight. By assigning 25 per cent of the counts injected to the

skeleton and assuming the value of S.R. to be that of plasma the concentration of sodium was calculated to be 71 m.eq. per kgm. of skeleton. This concentration, no doubt fortuitously, is exactly that reported by Harrison et al. (11).

b. *Rate of penetration into tissues.* Since radioactive sodium in one hour leaves the plasma and becomes distributed throughout tissues, in the manner already described, the first interpretation suggested is that all tissue sodium in the body is extracellular and in free diffusion with plasma sodium. On this basis nervous tissue exhibits a certain degree of impermeability. That it is not complete but merely a relative impermeability causing delayed entrance is to be expected, and is clearly demonstrated by allowing longer periods of time for penetration than one hour (tables 1 and 2). Since the completion of this work Hahn, Hevesy and Rebbe (9) have published analyses on rabbit tissues 67 hours after injection. The value of $(H_2O)_E Na^{24}$ calculated from their data is 31 grams, which although chemical sodium was not determined cannot differ greatly from $(H_2O)_E Na$.

This relative impermeability of brain to injected sodium suggested that if tissues had intracellular sodium phases, differential rates of entrance into them might exist. Whereas 3 to 12 hours were required for Na^{24} to enter brain it might enter the sodium-containing phase of other tissues much more rapidly. In fact it should diffuse almost instantaneously into the extracellular phase and, although delayed in entering cells, this too might be complete in an hour. Experiments were designed to determine the rate of entrance into tissues in shorter periods of time than 1 hour. Penetration was taken to be complete when the Na^{24} ratio equalled the Na ratio, and as an initial premise a delay in attaining completeness was considered indicative of an intracellular sodium fraction.

Rabbit 2 was decapitated in 13 minutes after injection and the results are given in column (8) table 1. The situation is somewhat complicated in this animal by the fact that complete equilibrium was not yet attained. Plasma samples were analysed at 3 minute intervals during this period to follow the rate of disappearance of radioactivity. Although the curve showed a tendency to flatten out in 13 minutes (see Griffiths and Macgraith, (8) who report constancy in 15 to 20 minutes), nevertheless 20 per cent of the dose injected was still in the plasma and the per cent distribution was appreciably lower than in rabbit 1. The penetration seemed to be less complete in certain tissues of rabbit 2 than of rabbit 1, notably in testes and brain.

In order to obviate individual differences, the next animal (rabbit 3) was anesthetized and the same tissue sampled over a period of 3 hours. The operative procedure was considered too drastic to keep the animal in the best physiological condition, but nevertheless some of the results are worth recording. There were indications that more Na^{24} had entered

skin, tendon, kidney, liver and muscle in $1\frac{1}{2}$ hours than in 17 minutes. The following figures serve to confirm and extend those in table 1: In liver in 1 hour the Na^{24} and Na ratios were 18 and 19 respectively; in perirenal fat 14 and 12; in 3 hours the Na^{24} ratio in cartilage was 93, in gastrocnemius 12, in cerebrum 29 and in ovaries 29. From this one result Na^{24} seems to be delayed in its entrance to ovaries because the Na ratio calculated from the chemical sodium analyses of Manery and Hastings (18) is 35.

This rabbit was pregnant and because relatively few studies of electrolytes in the *reproductive organs* have been carried out some of these tissues, were analysed for radioactivity, sodium and potassium. In 3 hours after injection the values of the so-called extracellular water based on Na and on Na^{24} were 55 and 52 grams respectively in uterine muscle, 60 and 22 grams in the endometrium, and 52 and 9 grams in the embryos. As we have stated the animal was in rather poor condition but in this particular case Na^{24} penetrated uterine muscle but did not pass through the endometrium into the embryos. These tissues differ from most in containing rather high concentrations of both potassium and sodium. The sodium concentrations in uterus muscle, endometrium and embryos were 84, 93 and 79 m.eq. per kgm. of fresh tissue and the potassium concentrations were found to be 72, 50 and 60 m.eq. per kgm. in the corresponding 3 tissues.

Rats injected intraperitoneally were next used. In animals intravenously injected, the plasma count is falling with time until equilibrium is reached, whereas in the intraperitoneal injections the per cent of the dose injected remains relatively constant in the plasma (see table 2), Na^{24} being absorbed from the peritoneal cavity at about the same rate as it leaves the circulatory system to enter the tissues. However, contamination of abdominal tissues with the intraperitoneally injected fluid can give rise to considerable error in short time experiments. Care was taken to minimize this source of error by removing the capsule of the kidney, sampling the liver from the interior, etc.

The results are summarized in table 2. In rats, too, we find the extracellular water based on Cl to be somewhat larger than that based on Na in some tissues, notably testes, stomach wall and liver whereas the sodium value seems to be slightly larger in abdominal, gastrocnemius and heart muscles. The analytical sodium and chloride concentrations in each tissue may be calculated by substituting the plasma concentration and the value of $(H_2O)_E$ Cl and $(H_2O)_E$ Na in equations (1) and (2).

The most complete data were obtained in rats 10 and 11, which show that in 3 hours excellent agreement was obtained between the Na and Na^{24} ratios in all tissues except testes and brain where the difference is 20 per cent, while in 12 hours the difference in testes seems to have disappeared

while that in brain persisted. In spite of the omissions the tissues can be divided into at least 2 groups; *a*, those in which the penetration of Na²⁴ is complete in 20 minutes and remains constant for at least 12 hours, and *b*, those into which the penetration is delayed but gradually proceeds to completion in 3 to 12 hours.

TABLE 2

Values of $(H_2O)_E$ for tissues of rats killed at varying times after intraperitoneal injections of Na^{24}

TISSUE	RAT 1		RAT 2		RAT 3	RAT 4	RAT 5	RAT 6	RAT 7		RAT 8		RAT 9	RAT 10		RAT 11
	359		294		189	180	190	155					153	245		205
	3 min.		8 min.		8 min.	16 min.	20 min.	1 hr.	1 hr.		2 hr.		2 hr.	3 hr.		12 hr.
	Cl	Na²⁴	Na	Na²⁴	Na²⁴	Na²⁴	Na²⁴	Na²⁴	Cl	Na²⁴	Cl	Na²⁴	Na²⁴	Na	Na²⁴	Na²⁴
Skin................	44.1	27	40.9	19	34						40.9	39		39.2	41	58
Abdominal muscle..	14.0		18.5								16.2	22		18.1	19	
Kidney.............	40.4		37.3			46*	43*	41*	55.5		48.6	49	39*	38.8	41	44
Liver..............	20.7		18.3			20*	19*	19*	24.0	19	25.4	18	19*	17.7	19	20
Testes.............	56.3		30.6	22	20	14*	15*	21*	57.2	20		22*		30.7	25	29
Gastrocnemius......	10.5	7.1	13.4	8.7	11	13*	11*	15*	12.3	14	12.1	15	15*	14.3	13	15
Gastro-intestinal tract...............	53.4		31.8						32.4†	29				34.4	31	29
Heart...............	22.2		35.7	33	12									31.6	31	27
Femur...............	20.8	7.2			33											66
Brain...............		3.1	30.2	5.5	1.9									31.5	25	24
Plasma Cl (m.eq. per l.).............	113.2															
Plasma Na (m.eq. per l.).............			142.0													
Na²⁴ perit. fluid / Na²⁴ (H₂O)ₑ	14.3		4.7		3.0	2.0	1.6		1.1		1.1			0.86		
Per cent distribution‡..............			29		29	27	28		28		30			32		
Per cent dose in plasma............	4.2		6.9		9.2	8.2	8.1	8.0	8.9		8.3		5.5	8.0		6.8

Tissues of rats 2 and 10 were ashed in muffle furnace, those of other animals in nitric acid on a steam bath.

* Averages of duplicate analyses.

† Tissue analysed is the wall of the small intestine. Other figures in this row represent analyses of stomach wall.

‡ For the calculation of these values it was assumed that no Na²⁴ was excreted.

We are indebted to Dr. W. O. Fenn for experiments on animals 7 and 8.

Skin, kidney, liver and gastrocnemius muscle unquestionably belong to group *a* and, while the data are rather incomplete, it is probable that abdominal muscle, heart and the gastro-intestinal tract should likewise be included. There is no evidence of an accumulation of Na²⁴ in heart

such as that reported by Griffiths and Macgraith (8). In group *b* are found testes, brain, and probably femur.

The values of the Na²⁴ ratios for *liver* are remarkably consistent and always equal to the sodium ratio but lower than the Cl ratio. There is some evidence that liver cord cells do not contain chloride (20) but it seems reasonable to suppose that Kupfer cells and others of connective tissue origin may have an intracellular chloride fraction without a corresponding sodium complement (see 17). In this tissue, then, the calculation of extracellular water based on sodium is probably a more accurate representation of its true volume than that based on chloride.

The intracellular sodium fraction, which has been postulated to exist in mammalian *muscles*, is so small that it is hardly detectable by the radioactivity method as used here. This method will not detect differences of less than ± 7 per cent. However, the values of extracellular water based on Na²⁴ in gastrocnemius muscles are somewhat less in rats 1 to 5 than in rats 6 to 11. There is some indication that only the chloride space was entered in 20 minutes and that more time was required to penetrate the entire sodium space. A careful investigation is necessary in which chloride, sodium and Na²⁴ are more accurately determined on the same muscle. One conclusion is clear-cut, namely, that if there is any intracellular sodium in muscle it comes into equilibrium with plasma sodium in at least one hour. In this connection the experiments of Heppel (13) are pertinent. He injected radioactive sodium into animals raised on a potassium deficient diet and found that it quickly penetrated the extracellular phase of the muscle as measured by chloride, and then slowly entered the muscle fibres for 1 hour at which time it had penetrated the volume occupied by chemically determined sodium. The most reasonable explanation at the moment of these findings is that sodium has penetrated muscle fibre membranes hitherto believed impermeable to it.

Kidney, which likewise belongs to group *a*, deserves special mention because, although Na²⁴ penetrates the entire sodium-containing portion very rapidly, it is likely that the tissue contains both intracellular sodium and intracellular chloride (5). The active reabsorption thought to occur in the distal tubules would be difficult to explain if it were otherwise. The rapid rate of entrance may result from the high speed of turnover of threshold substances which must indeed occur in view of rapid blood flow through the kidney.

Brain, peripheral nerve, testes and femur constitute group *b*, where there is unmistakably a delayed entrance of radioactive sodium into the sodium space. The question is raised as to whether this delayed entrance is a true manifestation of intracellular sodium.

Brain has a relatively high sodium content. If it were all extracellular the calculated extracellular fluid would be 30 to 35 per cent. But prac-

tically no Na²⁴ had entered this area in 8 minutes and in both rats and rabbits the amount penetrating had increased but penetration was not complete even in 3 hours.   Other demonstrations of the existence of a relatively impermeable barrier between plasma and brain tissue have been reported.   Wallace and Brodie (21, 22) demonstrated that the administered anions, iodide, thiocyanate and bromide were distributed from the plasma into tissues in the same fashion as chloride in every tissue studied except brain.   They found by direct analyses (23) that these anions had not entered the cerebrospinal fluid.   Furthermore the ratio of chloride to administered anion for the tissue of the central nervous system was always the same as that in the cerebrospinal fluid while the ratio in liver and lung corresponded to that in the plasma.   This was confirmed for bromide by Weir and Hastings (24).   The hypothesis presented by both groups of authors that the cerebrospinal fluid represented the extracellular fluid of nervous tissue seems plausible.

The cerebrospinal fluid was not analysed for radioactive sodium in our experiments, but in the light of the work just discussed it may have been retarded in entering this fluid.   Its behaviour simulates that of bromine and iodine (23), being greatly delayed but gradually entering the tissue in the course of several hours.   We conclude that sodium injected into the circulatory system does not diffuse freely into the sodium phase of the brain.

Very little is known about electrolytes in the reproductive organs. *Testis* is like gastric mucosa in showing a chloride concentration in excess of sodium (see also 18).   The sodium gives a more reasonable value for the extracellular fluid than the chloride, but Na²⁴ enters only two-thirds . of the total sodium space in 20 minutes and seems to be delayed in its entrance into the other third.   There are two main types of cells in the testis, connective tissue of which the interstitial cells may be secretory, and cells of the seminiferous epithelium in the tubules which is composed largely of sex cells.   The tissue is glandular and our present information will only permit conjecture.   Possibly the delayed entrance is due to secretion which might be much slower than in the kidney, for example, or perhaps circulatory differences are partially responsible.

The data on *bone*, although somewhat sparse, are of interest because they illustrate another case in which delayed entrance of Na²⁴ does not necessarily indicate intracellular sodium.   The value of $(H_2O)_E$ Na calculated from the data of Harrison et al. (11) is 50 grams.   Hence Na²⁴ has not entered the entire sodium containing area in 8 minutes, although it may have done so in less than 12 hours in view of the completion of the penetration into cartilage in 1 hour (table 1).   Harrison (10) found all the chloride of bone with its sodium complement in the organic phase.   The excess sodium was associated with the inorganic part of the interstitial substance.   The

rate of penetration of Na^{24} in this case may indicate the rate at which sodium is renewed in the inorganic compound of which it is a part.

c. *Discussion* of 1. In order to give these data some semblance of orderliness we have commenced with the initial premise that slow entrance of radioactive sodium into tissues is a manifestation of intracellular sodium. It was obvious at the outset that such a simple postulate would be quite inadequate. Radioactive sodium will certainly pass almost instantaneously from the plasma into areas which are in free diffusion equilibrium with plasma. Taken in conjunction with other evidence the rapid entrance into liver and muscle undoubtedly indicates the extracellular position of most of the sodium in these tissues. But it is not necessarily true that all areas into which penetration is rapid are extracellular. The kidney is a case in point providing the interpretation suggested above is tenable. Similarly, slow penetration may not always demonstrate intracellular sodium. This seems to be the case with brain and bone.

As more and more data accumulate it becomes increasingly evident that generalizations which embrace all tissues cannot be made with regard to electrolytes. Every tissue is a composite of many types of cells, each with a specific function and on this function the electrolyte distribution ultimately depends.

We may conclude from these data 1, that radioactive sodium will penetrate the extracellular phase of tissues very rapidly; 2, that in the case of secretory cells it may quickly enter an intracellular phase; 3, that a delayed entrance may demonstrate the presence of an intracellular phase, the entrance into which is controlled by intracellular processes, or it may illustrate the interposition of a barrier between the blood and the extracellular phase of certain tissues, e.g., brain.

2. *Na^{24} versus P^{32}*. Another method of elucidating the position of sodium in the animal organism is by comparing its behaviour to that of phosphorus which is known to be essentially an intracellular ion. Although several investigations have been published on the distribution of radioactive phosphorus in tissues (see (14) for review), none present suitable data for our purpose because, in general, the whole blood and not the plasma has been analysed for radioactivity. We have calculated (see table 3) the tissue:plasma ratio for P^{32} according to equation 4, and called it $(H_2O)_E$ P^{32}, though obviously the symbol can have no real meaning when applied to an intracellular ion.

Radioactive phosphorus differs from radioactive sodium in regard to its distribution (tables 2 and 3). In the case of Na^{24} about 8 per cent of the dose administered intraperitoneally is found in the plasma in 8 minutes and this remains constant over several hours while the amount of P^{32} in plasma gradually decreases with time. P^{32} is probably subjected to meta-

bolic influences which produce a continual drain on plasma phosphorus. Plasma sodium, on the other hand, comes into equilibrium with a more or less finite volume which does not vary with time, and which therefore can most reasonably be identified with the morphological extracellular phase. Similarly the percentage of the body weight into which the administered dose becomes distributed, i.e., per cent distribution, is constant at about 30 per cent for Na²⁴ but increases with time in the case of P³² exceeding 100 per cent in 20 minutes. Again the participation of phosphorus in intracellular processes is demonstrated.

TABLE 3

Calculations pertaining to rats injected intraperitoneally with radioactive phosphorus

TISSUE	RAT 12		RAT 13	RAT 14		RAT 15	
	7 min.		14 min.	20 min.		2 hrs.	
	Values of $(H_2O)_E$ (grams per 100 grams fresh tissue) calculated from						
	Cl	P³²	P³²	Cl	P³²	Cl	P³²
Skin........................	51.4	33	29	45.2	38	51.5	98
Kidney.....................	41.8		104	48.5	242	45.3	787
Liver......................	23.5		90	30.3	193	26.1	1,007
Testes.....................	51.4	58	56	55.0	30	52.9	50
Gastrocnemius.............	12.2	12	10	14.6	12	14.0	73
Stomach...................	58.8		89	53.3	124	51.5	388
Heart.....................	24.7		48	28.6	65	23.6	265
Femur.....................	24.9	36	61				
Brain.....................	29.3	Negl.	Negl.	30.0	Negl.	30.9	Negl.
$\dfrac{\text{P}^{32}\text{ peritoneal fluid}}{\text{P}^{32}\ (H_2O)_E}$	28.9		23.6	13.9		3.8	
Per cent distribution.......			58.6	118		377	
Per cent dose in plasma....	2.3		3.0	1.5		0.6	

Negl. = negligible.

Examination of individual tissues shows a fundamental difference between Na²⁴ and P³². In general, contrary to the behaviour of Na²⁴, the concentration of P³² in tissues rises with time, the value of $(H_2O)_E$ P³² approaching or exceeding 100 per cent.

As in the case with sodium there is a differential rate of entrance of P³² into tissues. The volume entered exceeds the $(H_2O)_E$ Cl in 14 minutes in heart, liver, kidney, stomach wall and femur and gradually increases to varying amounts in 2 hours. The gastrocnemius muscle is of some interest because the P³² ratio in 20 minutes is almost exactly equal to the chloride ratio, while this is far exceeded in 2 hours. Surely this is a manifestation of the penetration of inorganic phosphate into muscle fibres. The entrance

is less rapid into skin, testes and brain. In testes in 2 hours only a volume equal to $(H_2O)_E$ Cl has been entered. In the case of brain one would offer the suggestion that P^{32} is also retarded from entering the cerebrospinal fluid and tissue spaces at the hemato-encephalic barrier.

An attempt was made to observe the entrance both in vivo and in vitro of Na^{24} into the *red cells* of 3 rabbits and 5 of the rats studied. The sodium concentration was found to be low in the erythrocytes of these animals, being 20 m.eq. per liter of cells in the case of the rabbits and 17 in rats values which correspond satisfactorily with those of 16.0 and 12 m.eq. per kgm. of rabbit and rat corpuscles respectively, found by Kerr (15). In both cases the figures are calculated from analyses of plasma, whole blood and hematocrit values. The cell sodium per liter of cells amounts to only about 14 per cent of the plasma concentration which, because of the error in hematocrit measurements and radioactivity estimates, is close to the limits of our determinations. To avoid using hematocrit figures S.R. (count per m.eq. $\div 10^3$) values were calculated for whole blood and plasma, and the ratio of Na^{23} in whole blood:Na^{23} in plasma compared to the ratio of Na^{24} in whole blood:Na^{24} in plasma. Instead of the two values of S.R. being equal and the ratios being the same as would be the case if complete exchange of Na^{24} with the intracellular sodium had occurred the differences ranged from 0 to 8 per cent with no consistent change with time. Hence the experiments were inconclusive with regard to the exchange of Na^{24} with the small amount of intracellular sodium present in these cells.

Dog erythrocytes, which were found to contain 107 m.eq. per liter of cells, were then studied in *in vitro* experiments. Five milliliters of whole blood were mixed with 1 ml. of strongly radioactive isotonic saline. Whole blood and plasma were analysed for radioactivity and sodium, and the unwashed cells for radioactivity. In 18, 36 and 64 minutes the cell counts were represented by 126, 175 and 219 respectively showing a gradual penetration of Na^{24}. In 18 minutes the value of S.R. was 2.3 for plasma and 1.7 for whole blood. If complete exchange of Na^{24} for the intracellular sodium had occurred these values would be equal. The ratio of Na^{23} in whole blood:Na^{23} in plasma was 0.9 while a similar ratio for Na^{24} was 0.7. This difference may indicate that all but 20 per cent of the cell sodium exchanged with Na^{24}. The work was not continued because of the appearance of a paper by Cohn and Cohn (3). However these authors washed dog erythrocytes with isotonic sodium chloride after exposure to radioactive solutions and did not include chemical sodium analyses. Using our value of 107 m.eq. per liter of cells the ratio of Na^{23} in cells:Na^{23} in plasma $\times 100 = 76$ whereas their corresponding ratios for Na^{24} did not exceed 65, indicating again that about 15 per cent of the red cell sodium had not exchanged with plasma sodium.

Again the comparison of the penetration of Na^{24} with that of P^{32} is of interest. A typical *in vitro* experiment with P^{32} using rabbit cells, carried out in the manner described above, shows the plasma count to decrease with time while the count of unwashed cells increased in the following manner: in 22 minutes 984, in 1 hour 2120, and in $3\frac{3}{4}$ hours 6500. Cells which were washed after $3\frac{3}{4}$ hours' exposure to radioactivity counted 4770. As in the case of Na^{24} the gradual penetration of radioactive phosphorus into red cells is illustrated.

3. *The use of Na^{24} to measure extracellular fluid.* The volume into which the absorbed radioactive sodium is distributed (in per cent of the body weight as calculated by equation 5) gives a value of 25 in rabbit 1 (table 1) and 29 in rats 2–8 (table 2). These are the same order of magnitude as those reported by Harrison et al. (11) who based their calculation on the assumption that all of the body chloride is extracellular, and are similar to the results obtained with sulfocyanate injections by Crandall and Anderson (4).

The term extracellular fluid should refer to the fluid in the interstitial spaces bathing tissue cells. But most of the methods used depend on the distribution of substances which enter the Cl-containing phase of tissues, and hence are in error by the amount of intracellular chloride present. Although injected Na^{24} eventually enters the entire Na phase of all tissues, the rapidity with which it is distributed throughout 29 per cent of body weight without further change in 2 hours strongly indicates that it diffused immediately into the true extracellular space. The following advantages seem to favour the Na^{24} method; 1, it can be quickly and accurately determined in very small amounts and in coloured solutions and would be particularly suited to simultaneous measurements of plasma and extracellular fluid volumes; 2, excretion will be slight in the short time required to enter the extracellular fluid; 3, Na^{24} will not enter erythrocytes in those animals whose red cells are high in potassium and it can easily be determined when the cells contain sodium instead of potassium; 4, most important of all, Na^{24} can be administered in a few milliliters of isotonic saline and not only is this solution non-toxic but it obviates the need of introducing any substance in abnormal amounts.

SUMMARY AND CONCLUSIONS

1. The distribution of administered radioactive sodium (Na^{24}) throughout mammalian tissues was compared to that of normally occurring sodium (Na) and chloride (Cl). The method depended on the comparison of the ratios of the tissue:plasma concentration for each substance.

If sufficient time elapsed (up to 12 hrs.) the Na^{24} distribution was identical to the Na distribution, showing that there was no complete

impermeability to injected sodium in tissue phases which already contained sodium.

2. Tissues differed in the rates at which Na^{24} entered the Na phase. In general, it penetrated the extracellular phases rapidly and the Na-containing intracellular phases more slowly, penetration of the latter being clearly demonstrated in dog erythrocytes. Entrance proceeded rapidly into the Na phase of skin, kidney, liver and muscles but was delayed in testes, femur and brain. The significance of these findings with regard to the intra- or extracellular sodium of specific tissues has been discussed.

3. Na^{24} slowly entered dog erythrocytes which contain sodium but could not be conclusively demonstrated to penetrate rabbit or rat red cells which have a low sodium concentration.

4. Many of the differences found between the distribution of Na^{24} and P^{32} illustrate the fact that sodium is essentially an extracellular ion while phosphorus is concentrated within cells. However the slow penetration of both into nervous tissues indicates that certain barriers may be imposed between the blood and the extracellular phase of tissues, and that the position of a substance as intra- or extracellular is not the sole determining factor.

5. The extracellular fluid as measured by radioactive sodium was 29 per cent of the body weight in rats.

6. Some sodium, potassium and chloride analyses of reproductive organs and embryos are included.

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THE ELECTRICAL RESPONSE OF THE KITTEN AND ADULT CAT BRAIN TO CEREBRAL ANEMIA AND ANALEPTICS[1]

B. LIBET, J. F. FAZEKAS AND H. E. HIMWICH

From the Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, New York

The present report is concerned with a comparative study of the kitten and the adult cat brain to cerebral anemia under varying conditions which include 1, the effect of analeptics on cerebral electrical potentials; 2, the sensitivity of spontaneous cortical activity to cerebral anemia as compared with that of the evoked acoustic cortical response, and 3, the age of appearance of both spontaneous and evoked potentials.

METHOD. Silver-silver chloride wick electrodes (1) were used, a monopolar lead on the cerebral cortex and the other indifferent one on the frontal bone, feeding a push-pull amplifier and inkwriter (Grass). The cortex was exposed under ether, the animal curarized and placed under artificial respiration and the ether was blown off before electrical activity was recorded. In some experiments a 10 per cent solution of metrazol was injected intravenously in doses varying from 0.05 to 0.6 cc. In other observations a saturated solution of strychnine was painted on the cortex. To determine the effects of sudden brain anemia on the persistence of electrical activity, the heart was quickly excised from the previously opened thorax.

RESULTS. *Normal spontaneous activity and age.* In the 2 to 6 day old kittens, electrical activity of the cerebral cortex is practically absent or of very low amplitude (5–20 microvolts). When present this activity consists of random waves. Nevertheless in these kittens electrical responses from the cortex in the region of the ectosylvian sulci can be evoked by auditory stimuli, namely, a sharp noise. Nine day old kittens exhibited random slow waves from the occipital and frontal regions, but considerable activity or irregular frequencies (amplitude about 50 microvolts) from the regions about the anterior and posterior ectosylvian sulci. From these areas, as in the younger kittens, electrical responses to auditory stimuli can be readily elicited. Only part of the spontaneous activity of this region may be due to background noise; for example, the spontaneous

waves disappear approximately 15 seconds after complete cerebral anemia while responses to acoustic stimuli can still be evoked at this time and these responses are of the same or larger amplitude than those before cerebral anemia. Kittens, 19 to 25 days of age, exhibit considerable electrical activity in the same region with an amplitude varying from 20 to 100 microvolts, and a frequency from 1 to 35 per second (fig. B1). Though the faster components are prevalent, they are more irregular and slower, 21 per second, than those appearing in the adult, 40 per second. Compare figure 1, A1, B1 and C1, normals for a 3 day and a 25 day old kitten and an adult cat respectively.
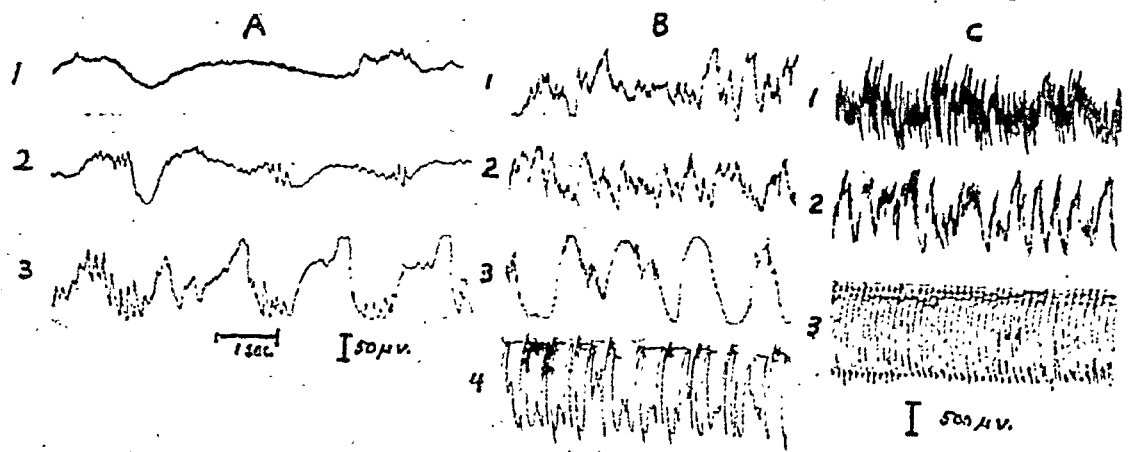

Fig. 1. Normal and metrazol activity of cerebral cortex in kitten and adult cat.
A. Three day old kitten. *1.* Spontaneous activity. *2.* After 0.1 cc. of 10 per cent metrazol intravenously. *3.* After another 0.1 cc. metrazol.
B. Twenty-five day old kitten. *1.* Spontaneous activity. *2.* After 0.1 cc. metrazol intravenously. *3.* After another 0.17 cc. metrazol. *4.* Another 25 day old kitten, 0.4 cc. metrazol into heart (had received 0.13 cc. metrazol into heart previously, which produced slow waves, resembling those in B2 here).
C. Adult cat. *1.* Spontaneous activity. *2.* Response after 0.2 cc. metrazol intravenously. *3.* Another adult cat—0.5 cc. metrazol intravenously.
All same amplification as shown below A3 (except C3—see below it).

Effect of metrazol and strychnine. The response to metrazol varies with the dose and the age of the animal. In the relatively silent cortex of 2 to 6 day old kittens electrical activity can be initiated by the injection of metrazol or strychnine, or the local application of the matter. In these newborn animals the intravenous injection of 0.05 cc. of metrazol usually resulted in sporadic bursts of activity for approximately 100 seconds. These often consisted of rather regular waves from 9 to 12 per second (20–50 microvolts) interspersed with spikes and slower, often diphasic components (fig. 1, A2). Larger doses of metrazol between

0.1 and 0.2 cc. initiated a more continuous activity of somewhat greater amplitude (fig. 1, A3). In many instances trains of large polyphasic waves appeared. The results with strychnine were similar to those observed with metrazol. In the 19 to 25 day old kitten, the injection of 0.1 cc. of metrazol accentuated the slow waves (fig. 1, B2). These were enhanced by a larger dose of 0.17 cc. (fig. 1, B3). Another kitten (fig. 1, B4) receiving a convulsive dose of 0.4 cc. of metrazol produced a train of spike-like waves similar to the adult but of lower frequency. Compare figure 1, B4 and C3.

The amounts of metrazol required to produce the central electrical responses or the peripheral motor convulsions were approximately the same, 0.15 cc. in 2 to 5 day old kittens. This is also true in the adult dog (2) and in humans (3). Like the electrical responses, the motor convulsions in the 4 day old kitten appeared to be different in nature from those of the adult. Instead of the violent clonic convulsions observed in the adult with metrazol, the kitten exhibits slower and smoother generalized movements of the limbs and trunk.

Cerebral anemia. The persistence of cortical electrical activity after rapid extirpation of the heart (an operation requiring less than 3 seconds) could be studied in the 2 to 6 day old kittens only after some electrical activity had been initiated by metrazol or strychnine. The survival time of such activity averaged 22 seconds. Though most activity disappeared by this time, in all cases occasional subsequent bursts of activity were noted from 39 to 100 seconds. After cardiac excision, the adult survival period, without the injection of metrazol, varies from 10 to 15 seconds. It does not appear probable that the longer survival time found in the 4 day old kitten, as compared with the adult, is due to use of metrazol. Older animals do not survive longer after receiving metrazol.

Evoked cortical response to acoustic stimuli. Bremer and Dow (4) have described the electrical response of the auditory area of the cat to acoustic stimuli. We have also observed responses to auditory stimuli in kittens (3-24 days old, as already mentioned above) and adult cats, with the active monopolar lead on the regions about the ectosylvian sulci. The responses of the older kitten and adult cat cortex to a sharp auditory stimulus (clap of hands) consisted usually of a surface positive wave (50-100 microvolts) about 0.05 second (0.03-0.15 sec.) duration (fig. 2). The disappearance of the response after a certain duration of cerebral anemia, despite repeated noises of the same or greater intensity, acted as an obvious control for other vibrations set up with each noise. Frequently, the response was diphasic with a surface negative phase following a surface positive one. In some cases the response consisted of an initially surface negative diphasic wave. Metrazol was found to enhance the magnitude of the response and to increase the number of after waves. Metrazol also caused

the appearance of the auditory response in adjacent cortical regions which had previously not shown it.

Evoked cortical response and cerebral anemia. Whereas the ordinary spontaneous electrical activity in the cat (about 19 days old and older) cerebral cortex disappears within about 15 seconds after initiating complete cerebral anemia, electrical responses of the acoustic cortex to auditory
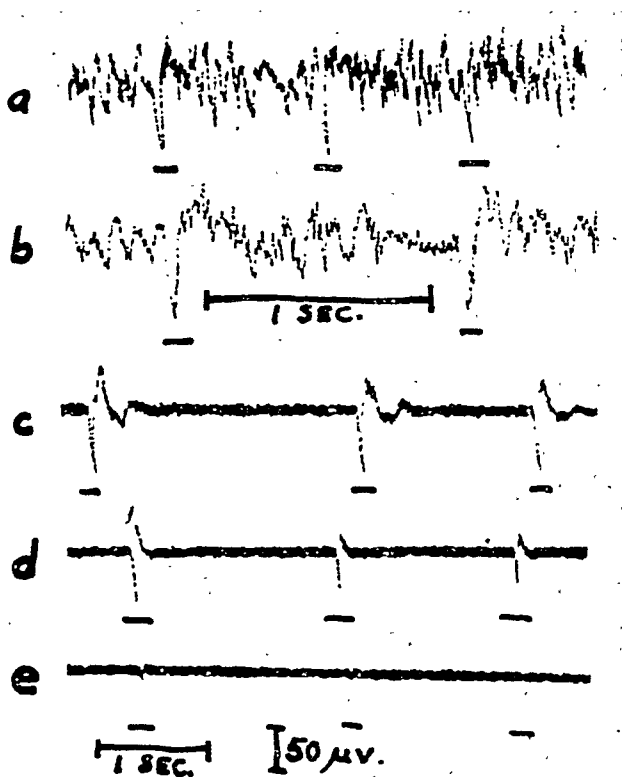
Fig. 2. Cortical acoustic response in cerebral anemia.

A. Normal spontaneous activity, 5 week old kitten cerebral cortex, from ecto-sylvian gyrus. Each mark underneath indicates a clap of hands. (These were marked down by hand, and the exact time of the sound cannot be told, i.e., latent period cannot be measured.) B. Same as A faster speed recording. C. Sixteen seconds after heart cut out. Spontaneous waves are already gone. D. Fifty seconds after heart out. E. Eighty seconds after heart out.

All (except B) at same speed recording (see below E).

stimuli can be evoked for as long as 50 to 100 seconds after complete cerebral anemia (fig. 2). All phases of the evoked response gradually diminish in amplitude, although sometimes an initial increase in magnitude occurs. In one case (fig. 2), a second small surface positive and negative wave which followed each initial response disappeared 45 seconds before the latter.

DISCUSSION. These results reveal that the characteristic adult activity
is not apparent in the newborn kitten. However, there seems to be a
gradual increase with age in the frequency, regularity, and amplitude of
the waves. Adult activity was not fully developed in 25 day old animals.
A similar development of the electroencephalograph of humans has been
described (5).

Since metrazol and strychnine can induce electrical activity in the
cerebral cortex of the infants, the neurones in the cortex of the kitten
possess the ability to produce electrical oscillations when influenced by
appropriate agents. These agents may act either by causing synchroni-
zation of waves already present asynchronously in the various units or by
altering the metabolism or membrane properties of the cells in such a way
as to initiate oscillatory activity. The ability of the 4 day old kitten
cortex to show electrical responses to acoustic stimuli, though it is com-
paratively lacking in spontaneous activity, indicates that there is already
present in the infant functional sensory transmission to the cortex.
Whether these electrical responses are post-synaptic or electrical changes
from presynaptic fibers to the cortical cells cannot be determined.

The activity induced in the kitten by metrazol is somewhat different
than that occurring in adult cats with or without metrazol. In the infant
both the regular rhythms and the large polyphasic convulsive type of
waves are slower than in the adult.

The doses of metrazol required to produce some slight change in the
electrical activity in the 2 to 6 day old, 19 to 25 day old kittens and the
adult cat were approximately 0.05 cc., 0.1 cc. and 0.2 cc. respectively.
Since the respective weights are approximately 100, 250 and 2500 grams,
it is apparent that the dose per unit of body weight is larger for the infant.
The doses necessary to produce the convulsive type of spike-like waves,
as well as convulsive motor activity, show a similar relationship to age,
namely, about 0.1, 0.3 and 0.6 cc. of metrazol respectively. Since the ratio
of the minimal convulsive dose of the infant, as compared to the adult, is
about 1 to 5 while the ratio of their body weight is about 1 to 25, the infant's
central nervous system is apparently less responsive to metrazol.

The results also indicate a greater resistance of the infant brain to
cerebral anemia and are in accordance with the observation on revival
ability of the central nervous system of puppies and adult dogs after cere-
bral anemia (6). It should be noted, however, that though the survival
time of the 2 to 6 day old kitten was somewhat longer than in the adult
that of the 19 to 25 day old kitten was not appreciably so. Kabat and
Dennis (6), on the other hand, found that puppies of 49 days of age could
be revived after longer periods of cerebral anemia than those which could
be sustained by the adult dog. This, with our finding on the survival time
of the 19 to 25 day old kitten, may indicate that revival time is not nec-

essarily a direct function of survival time. In seeking an explanation for these differences between the infant and adult brain, *in vitro* studies of their respiratory metabolism are suggestive. It has been observed that the cortex of infant rats, puppies and kittens possesses a lower metabolic rate than adults of these species (7, 8).

That the evoked cortical acoustic response can be elicited long after spontaneous electrical activity in the cortex has disappeared during complete cerebral anemia is highly interesting. It recalls the observation of Forbes and Morison (9) of evoked potentials elicited in deep barbiturate anesthesia when spontaneous waves had disappeared. However, these investigators could elicit their secondary discharge response, which resembles our evoked response, only after spontaneous activity had been abolished, while our acoustic response could be elicited in the presence of spontaneous activity as well as in its absence. On the other hand, Sugar and Gerard (10) found that spontaneous waves and evoked visual potentials in the lateral geniculate of the cat disappeared simultaneously (though later than cortical spontaneous waves) during complete cerebral anemia.

The observed evoked potential in response to an acoustic stimulus may be 1, a result of electrical changes in the cortical cells or dendrites responding to synaptic stimulation, or 2, an action potential of nerve fibers carrying impulses to the cortex, or 3, a combination of 1 and 2. The relatively long duration (about 0.05–0.15 sec.) and large size (50–100 microvolts) of the response waves as recorded from the cortical surface suggest the first possibility as the most probable one, though the second cannot be excluded. (See also the resemblance to the "deep response" obtained by Adrian (1) upon electrical stimulation of the cerebral cortex.) The sensitivity of spontaneous cortical activity to anemia (i.e., its disappearance within 15 seconds of complete anemia) has been attributed not to the nerve fibers but rather to the neurone units in the cortex (11), which presumably generate the spontaneous rhythms (12). If, then, the acoustically evoked potentials are also neuronal responses to synaptic stimulation then several interesting corollaries would follow as to 1, the relative sensitivity of synaptic transmission and spontaneous electrical activity to anemia; 2, a possible distinction between metabolism underlying the production of spontaneous activity and that underlying function in cell synaptic responses, and 3, a difference in the sensitivity of these metabolisms to anemia.

<div align="center">CONCLUSIONS</div>

1. A gradual development of the frequency, regularity, and amplitude of the cortical electrograms occurs during infancy as seen in kittens 2 to 6 days old, 19 to 25 days old and adult cats.

2. Electrical activity may be induced in the comparatively silent brain

of the 2 to 6 day old kitten either by metrazol or strychnine.   Acoustic stimuli can also evoke cortical responses.

3. The activity induced by metrazol in the kitten differs from that observed in the adult cat both as to central-electrical and peripheral–motor manifestations.

4. The relative dose of metrazol (per unit body weight) required to elicit an electrical or motor response is greater for the infant than the adult.

5. After sudden excision of the heart, the persistence of cerebral electrical activity is longer in the 2 to 6 day old infant than the adult.   By the 19th to 25th day this difference from the adult largely disappears.

6. During complete cerebral anemia, the electrical response of the acoustic cortex to a sharp noise can be elicited for as long as 50 to 100 seconds, at a time when spontaneous electrical activity has long ceased. The possible significance of this and the above phenomena is discussed.

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THE PROTHROMBIN CONCENTRATION IN THE BLOOD OF VARIOUS SPECIES[1]

ARMAND J. QUICK

From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee

From studies made with the quantitative method for prothrombin, which the author described in 1935, he (1) concluded that rabbit and dog blood contain several times more prothrombin than does human blood. This conclusion was further strengthened by the finding that an amount of heparin which completely inhibited the coagulation of human plasma, containing an excess of thromboplastin and an optimum concentration of calcium, was quite inadequate for preventing the clotting of rabbit plasma under identical conditions (2). It was found that the quantity of heparin which inhibited the clotting of undiluted human plasma could prevent the coagulation of rabbit plasma only when the latter was diluted with approximately four parts of prothrombin-free plasma. From this it was concluded that the prothrombin is roughly five times higher in rabbit than in human blood. Recently, however, Warner, Brinkhous and Smith (3) reported that with their quantitative method for prothrombin, which they developed contemporaneously (4) with the author's procedure, they found that the prothrombin was only slightly higher in rabbit and dog than in human plasma.

This marked discrepancy makes it desirable to investigate further the problem of the prothrombin content of various bloods, and to find if possible the reason for the differences in the results obtained by the two methods.

EXPERIMENTAL. *Quantitative determination of prothrombin.* The method employed in this study was the same as that which the author described for the clinical determination of prothrombin in human blood (5). The blood was obtained by venipuncture, and mixed immediately with sodium oxalate. One volume of 0.1 M sodium oxalate was added to 9 volumes of blood. It has recently been demonstrated that this amount of sodium oxalate will completely decalcify blood in less than one minute (6).

Thromboplastin. The most satisfactory known source of thrombo-plastin is rabbit brain. By dehydrating this tissue with acetone according to the author's procedure, (5), (7), a material is obtained which is not only highly active, but also uniform in potency. The preparations used in this investigation coagulated human plasma in 11 to 12 seconds and rabbit plasma in 6 seconds (when 0.1 cc. of plasma was mixed with 0.1 cc. of thromboplastin solution and 0.1 cc. of 0.025 M calcium chloride).

Thromboplastin has group rather than species specificity. Rabbit thromboplastin is specific for rabbit, dog, cat, lion, deer, horse, cow, human and many other bloods, but rather sluggishly activates the pro-thrombin of the guinea pig and of birds. The action of thromboplastin made from the brains of different animals and tested on the blood of these same animals is presented in table 1. It will be observed that chicken and

TABLE 1

Species specificity of thromboplastin

	CLOTTING TIME OF PLASMA IN SECONDS			
SOURCE OF THROMBOPLASTIN	Type of plasma			
	Chicken	Guinea pig	Rabbit	Man
Chicken brain..........................	11	40	28	45
Guinea pig brain.......................	30	16	22	35
Rabbit brain...........................	60	24	6	$11\frac{1}{2}$
Human brain...........................	240	33	10	19
Control (none added).................	240	60	80	120

The clotting time was determined by mixing 0.1 cc. of plasma with 0.1 cc. of 0.025 M calcium chloride and 0.1 cc. of thromboplastin emulsion.

guinea pig thromboplastin show their greatest activity when reacting with their own prothrombins.

In this present work only the bloods of animals were studied whose prothrombin is specifically activated by rabbit thromboplastin. Most attention was given to human and rabbit blood since the first contains a relatively low prothrombin concentration, while the latter has a very high level. To obtain plasmas containing prothrombin of varying con-centrations, normal oxalated plasma was diluted either with prothrombin-free plasma prepared by the author's aluminum hydroxide method (8) or with 0.85 per cent sodium chloride containing enough sodium oxalate to make a 0.01 M solution.

RESULTS. When the clotting times of progressive dilutions of recalcified oxalated rabbit plasmas (containing excess thromboplastin) were deter-mined, it was found that the 20 per cent solution had the same coagulation time as undiluted human plasma while a 10 per cent solution clotted at

the same rate as a 50 per cent dilution of human plasma and that the same ratio continued for further dilutions as seen in table 2. This clearly suggests that rabbit plasma contains roughly 5 times more prothrombin than is present in human plasma. Since rabbit plasma contains the highest known concentration of prothrombin, it should serve satisfactorily as the standard for expressing the prothrombin content of blood. By arbitrarily fixing the normal prothrombin content of rabbit plasma as 100, the prothrombin concentration of other bloods can be expressed. In figure 1 such a curve is presented which contains the prothrombin level of various species in terms of the normal concentration of prothrombin

TABLE 2

The relationship between the clotting time and the prothrombin concentration of rabbit and human plasma

RABBIT PLASMA PRO-THROMBIN	CLOTTING TIME	HUMAN PLASMA PRO-THROMBIN	CLOTTING TIME
per cent	*sec.*	*per cent*	*sec.*
100	6		
50	7		
40	8		
30	9		
25	$9\frac{1}{2}$		
20	11	100	$11\frac{1}{2}$
		80	$12\frac{1}{2}$
15	13		
10	15	50	15
		40	17
		30	19
5	$19\frac{1}{2}$	25	$21\frac{1}{2}$
4	26	20	25
2	37	10	39
1	55	5	65

in rabbit plasma. Rabbit and dog bloods contain the highest concentration of prothrombin while curiously the plasma of the cow has the lowest level. No explanation for these variations can be offered. It is obvious that the concentration is not influenced by the size of the animal, its dietary habits, or domestication. According to present knowledge, these variations are of no practical significance since the writer has shown that even human blood with its low prothrombin can lose almost 80 per cent of this clotting factor before a hemorrhagic state becomes manifest.

The curve expressing the relationship between the concentration of prothrombin and the clotting time (in the presence of optimal concentrations of calcium and thromboplastin) is a hyperbola. Theoretically the asymptotes of this curve are $x = 0, y = 0$, but practically it is rather im-

probable that a concentration of prothrombin can be found which will cause instantaneous coagulation. The curve presented in figure 1 should be considered a segment of the complete theoretical curve. In this segment the normal concentration of prothrombin in rabbit plasma is assigned as 100.

It is rather significant that if one plots the clotting times of plasma or fibrinogen using progressive dilutions of thrombin, a similar hyperbolic curve is obtained. By means of the writer's modification of Eagle's method (2) a thrombin solution can be prepared which is not only highly
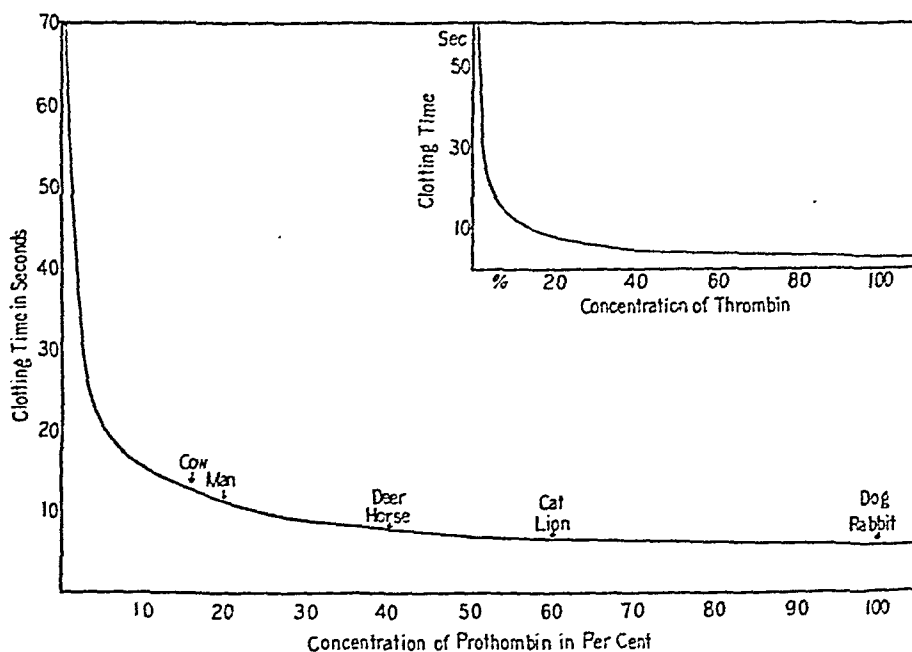

Fig. 1. The prothrombin concentration in the blood of various species. In the case of the lion and deer, determinations were done on only one specimen of blood. From preliminary work indications have been obtained that the prothrombin content of baboon blood is approximately the same as that of the human, but that the prothrombin of monkey's blood is higher.

active but is also remarkably constant in potency. When 0.1 cc. of this preparation is allowed to react with 0.2 cc. of oxalated plasma or a solution of fibrinogen, coagulation occurs in 3 seconds. By designating the concentration of this solution of thrombin 100, and determining the clotting times for a series of dilutions, the curve in the insert of figure 1 is obtained. The close similarity between the prothrombin and thrombin curves is additional evidence that the rate at which thrombin is formed is directly dependent on the concentration of prothrombin in the plasma, provided that the calcium and thromboplastin are kept constant.

COMMENT. From the results obtained in this study, one can conclude

that the prothrombin varies greatly in different species and that the blood of man contains only one-fifth as much prothrombin as rabbit or dog blood. Since these findings differ radically from those reported by Smith and his co-workers, it seems desirable to find the reason for this disagreement. Although it is asserted that Smith's method must remain the standard for the quantitative determination (9), certain observations are not in accord with this claim. It has not been demonstrated that the thrombin obtained in the final dilution of the 2-stage method is equal to the total that the prothrombin in the original plasma can yield. When Smith, Warner and Brinkhous improved their method, they were able to increase the units of prothrombin of normal dog blood from 200–325 to 325–400 (10). Thus, with only a small modification they increased substantially the yield of thrombin from the same amount of prothrombin.

If prothrombin is a pre-enzyme closely associated with plasma proteins, it is problematic whether such a substance can be diluted several hundred times without undergoing a certain amount of degeneration especially because of the disturbance of the protective colloids. A probable illustration of this is brought out in the determination of the prothrombin in the blood of newborn infants. Brinkhous, Smith and Warner (11) found that the prothrombin concentration was below 50 per cent of normal up to the age of two months and did not reach the adult level until the ninth month. The writer with Grossman (12) found, on the contrary, that the prothrombin was normal in infants except for a few days after birth. This short period of prothrombinopenia corresponds to the time when bleeding is frequently observed, and it can be prevented by means of vitamin K. Smith and his associates (13) believe that the prothrombin of the newborn is converted more readily to thrombin to compensate for the deficiency in quantity. While it is known that the speed of the conversion of prothrombin is influenced by the concentrations of calcium, thromboplastin, heparin and perhaps other substances, no direct evidence can be found that prothrombin itself can alter its convertibility. If the prothrombin is qualitatively different in infants, one must conclude that vitamin K increases the convertibility rather than the quantity of prothrombin. This, however, is not in accord with the results obtained using vitamin K in the prothrombinopenia of jaundice. In the latter condition all the results obtained both by Smith's and by the writer's method clearly indicate that vitamin K restores the prothrombin content of the plasma and does not influence the convertibility. It seems very likely therefore that the prothrombin of infants is qualitatively normal, but the plasma varying as it does from the adult, may not yield on dilution as much thrombin per unit of prothrombin as does adult blood. Likewise, it is probable that when the plasma of various species is diluted several hundred fold, and then treated with calcium and thromboplastin, the resulting mixture will represent an equilibrium of

thrombin, degenerated prothrombin and metathrombin rather than a solution of pure thrombin.

SUMMARY

By means of the method developed by the writer for the quantitative determination of prothrombin, it has been found that the concentration of this factor varies greatly in different species. If the prothrombin level of normal rabbit plasma is set at 100, the concentration found in various species is as follows: dog 100; cat 60; lion 60; horse 40; man 20; cow 16.

The probable reason why the 2-stage method of Smith for the determination of prothrombin fails to show this marked variation in prothrombin content of various bloods is discussed.

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THE EFFECT OF DENERVATION UPON THE RESPONSE TO ADRENALIN IN THE ISOLATED FISH SCALE MELANOPHORE

DIETRICH C. SMITH

With the technical assistance of RUTH ELLEN MUSSER

From the Department of Physiology, University of Maryland, School of Medicine, Baltimore, and Marine Biological Laboratory, Woods Hole

Adrenalin brings about rapid and complete concentration of melanophore pigment in teleosts either when injected or applied directly to the surface of the body. Microscopic examination reveals the cell in a typical punctate condition with the pigment solidly concentrated in the center. With the exception of sympathetic nerve stimulation no other agent is capable of producing so rapid and thorough a response. This fact has long been familiar to those working in the field of animal color changes. It has led to the view that the action of adrenalin is physiologically significant in melanophore responses and is probably involved in the normal paling of a fish upon a light background. This position is greatly strengthened when the rôle of the sympathetic nervous system in governing teleost color changes is considered. Control of melanophore activity in the bony fishes by the sympathetic nervous system has been thoroughly established by the work of Pouchet (1876), v. Frisch (1911) and many others.

Whether adrenalin plays in teleosts the same part in transmitting sympathetic nerve impulses from nerve ending to effector organ that it does in the higher vertebrates has not yet been demonstrated. It seems reasonable to assume that it does. If so it explains the great sensitivity of the melanophore to this substance. In the higher vertebrates the sensitivity of the effector organ to adrenalin is actually increased by denervation and subsequent degeneration of the nerve ending. This was first demonstrated by Meltzer and Auer (1904) and later by Cannon and Rosenblueth (1932) in the nictitating membrane of the cat. This bit of smooth muscle responds much more vigorously and exhibits a lower threshold to adrenalin after degeneration of the myo-neural junction than before. A similar phenomenon in the isolated scale melanophores of fishes would be of considerable theoretical interest.

That such a reaction does occur in the fish melanophore can be demonstrated. Scales from the common Tautog (Tautoga onitis) of the

Atlantic seaboard were selected for the experiment. Denervation of the scale was done in the following manner, the procedure being based upon a method devised for transplanting fish scales by Mori (1931) and Goodrich and Nichols (1933). A trunk scale was carefully pulled out of its scale pocket with a forceps, obviously severing all of its connections with the nervous and circulatory systems. The scale was then slipped back into the pocket and left there undisturbed. In a vast majority of cases the scales healed back in place, quickly established new circulatory connections and maintained themselves in a healthy condition. Nervous regeneration was characteristically slower, as shown by the failure of the scales to participate for some time in the normal color changes shown by the animal. Placed on a light background an operated fish would show dark splotches on an otherwise pale body due to patches of scales in which the denervated melanophores did not respond.

While release from central nervous control is immediate, independence of nervous influences is not. Even after removal of the scale from the body it still contains the severed distal ends of the nerve fibers and the nerve endings. The nerve twigs are only a millimeter or so in length and degenerate completely in about a day. Only when this occurs can the melanophore be said to be free from any nervous control. Once affected, however, any response exhibited by the melanophores of such a scale must be free of any nervous mediation whatsoever. The behavior of such preparations has previously been discussed (Smith, 1939). Indications are that regeneration of the nervous elements in the scale occurs in about two weeks. After this time the melanophores once more take part in the normal color responses of the fish to background, or concentrate when the pigment-motor center in the medulla is electrically stimulated.

The responses of these operated scale melanophores to adrenalin were studied during the period of denervation and compared to those in normal unoperated scales and those in scales showing recovery from denervation. The response was a simple one to obtain. The scale was removed from the side of the trunk and placed in a suitable quantity of balanced salt solution consisting of 6 vols. of N/5 NaCl, 1 vol. N/5 KCl, and 0.35 vol. N/5 CaCl$_2$. To this enough adrenalin chloride was added to make the adrenalin strength of the solution $\frac{1}{100,000}$. The scale melanophores exposed to this solution, either normal or denervated, showed the typical response to adrenalin, a rapid concentration of their pigment.

The speed of the response was measured photoelectrically by a method previously described (Smith, 1936). In brief it was as follows. The amount of light transmitted through an isolated scale was measured, the amount being a function among other things of the degree of melanin dispersion within the scale melanophores. As the melanophores concen-

trated their pigment more light passed through the scale with a consequent deflection of the galvanometer attached to the photocell.   Thus galvanometer deflection could be used as a sign of pigment movement.

The galvanometer deflections resulting from the pigment concentration produced by the action of adrenalin on each of ten different normal scales were recorded, the average deflection determined and plotted as shown in figure 1.   Several scales must be averaged because of variations in their size, thickness and number of melanophores.   Similarly the concentration of the melanophores in six scales denervated for four days and three scales denervated for six days was averaged and plotted.   Finally, the results obtained from six scales thirteen days after the operation were treated in the same way.   All four curves were plotted on the same graph as
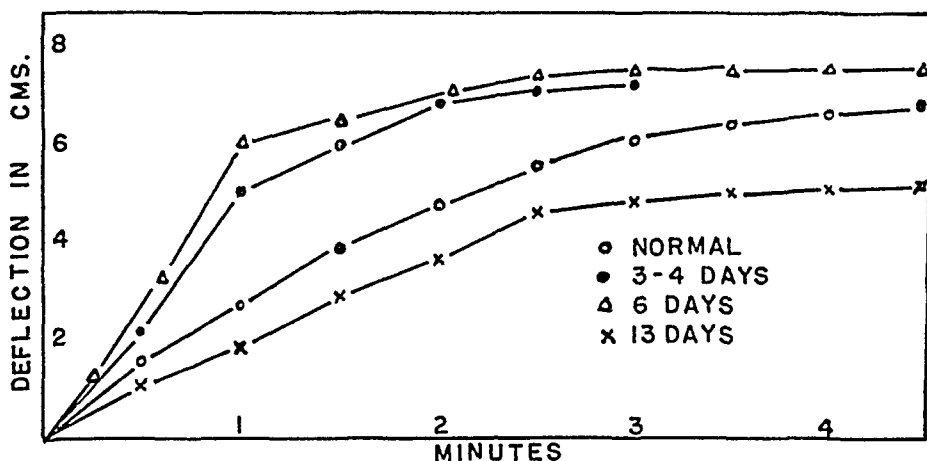

Fig. 1. Responses to $\frac{1}{100'000}$ adrenalin in isolated scales of the Tautog, both normal and denervated. Photo-electric recording.   Increase in galvanometer deflection indicates pigment concentration.

shown in figure 1.   From this it was clear that the scale melanophores four to six days after the operation concentrated their pigment about twice as rapidly as did those in normal scales.   Thirteen days after the operation the speed of the response was again the same as in the normal scales, indicating regeneration of the nervous elements lost as a result of the original operation.   Thus denervation and subsequent degeneration of the chromo-neural junction resulted in an increased sensitivity on the part of Tautog scale melanophores to adrenalin.   This established an essential similarity between the responses to adrenalin in fish melanophores and in the effectors of higher vertebrates where adrenalin is known to act as the mediator between the sympathetic nerve endings and the responding organ.   This does not of course prove that adrenalin so acts for teleost melanophores, although it does make it appear more likely.

Aside from any possible theoretical significance these results fully corrob-

orate the demonstration by Parker (1934) that adrenalin acts directly upon melanophores. He observed the familiar response to adrenalin in the tail melanophores of Fundulus after sectioning the nerves to the region involved and allowing ample time for the degeneration of distal nerve fibers and nerve endings. Prior to this demonstration Giersberg (1930) and others maintained that adrenalin acted upon fish melanophores only by way of the nerve ending and was incapable of affecting the cell directly.

The difference in timing between the responses of normal and denervated melanophores to adrenalin serves to explain a puzzling reaction observed some years ago in Phoxinus (Smith, 1931). Certain areas on the head of this fish were denervated by sectioning the ophthalmic nerve. Remarkably enough, if such operated fishes were placed upon a black background with skins uniformly dark and then subjected to a sudden frightening stimulus such as a sharp tap on the side of the aquarium the operated areas suddenly paled although the rest of the body remained black. At the time adrenalin release seemed the likely explanation for this reaction but it was difficult to see why the entire body was not affected. In the light of the increased sensitivity to adrenalin on the part of denervated melanophores the reaction is now easily explained.

SUMMARY

Isolated scale melanophores of the fish Tautog respond more rapidly to adrenalin after denervation than they do before.

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THE EFFECT OF PRIMING DOSES OF DESOXYCORTICOS-TERONE ACETATE IN PREVENTING CIRCULATORY FAILURE AND SHOCK IN THE ADRENALECTOMIZED DOG

W. W. SWINGLE, H. W. HAYS, J. W. REMINGTON, W. D. COLLINGS[1] AND W. M. PARKINS

From the Section of Physiology, Biological Laboratory, Princeton University, Princeton, New Jersey

Active vigorous adrenalectomized dogs kept in normal condition by daily injections of maintenance doses of cortical extract are extremely susceptible to any procedure which throws a strain upon the peripheral circulation. Trauma to muscle masses, intestinal manipulation, intravenous injections of massive doses of epinephrine, or intraperitoneal injection of isotonic glucose invariably induce circulatory collapse and shock within 5 to 15 hours. The same degree of trauma or quantities of injected epinephrine and glucose cause no untoward symptoms in the animal possessing adrenal glands. Likewise, circulatory failure does not develop if the adrenalectomized dog is primed with large doses of cortical extract just previous to the shock inducing procedures. It has been demonstrated, moreover, that if the animals are permitted to develop circulatory failure and shock, they can be readily revived and restored to normal by injections of adequate amounts of cortical extract (1, 2).

Since synthetic D.C.A. (desoxycorticosterone acetate) will maintain adrenalectomized dogs and rats (3–7) and is said to have a prophylactic action in human surgical shock and analogous conditions where circulatory failure is a prominent symptom (8–10), it was considered worth while to study its effect upon experimentally induced circulatory failure in the adrenalectomized dog.

METHODS. The adrenalectomized dogs employed had been operated for three months to one year before use, and kept in normal health by daily injections of maintenance doses of cortical extract. These dogs were active, vigorous, and at peak weight. Earlier studies by two of the writers (2) have demonstrated that in this type of animal the arterial pressure, serum electrolyte concentrations, blood sugar level, blood urea nitrogen concentration, water balance and renal excretion of electrolytes

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[1] E. R. Squibb and Sons Fellow in the Biological Sciences.

are normal as compared with nonadrenalectomized dogs maintained under similar laboratory and dietary conditions.

The method used for inducing circulatory failure and shock were: 1, the intraperitoneal injection of an isotonic glucose solution; 2, the injection of large amounts of epinephrine; 3, trauma to muscle masses, and 4, manipulation of the small intestine.

A dose of 60 cc. per kgm. body weight of a 5.5 per cent glucose solution was injected intraperitoneally without use of an anesthetic to induce glucose shock. Since extensive experimentation has shown that the circulatory failure in the adrenalectomized animal occurs independently of the presence or absence of the peritoneal fluid (11–12), paracentesis was not performed.

In the epinephrine experiments, a 1-5000 solution in 0.9 per cent saline of adrenalin hydrochloride (Parke, Davis and Co.) was injected into the saphenous vein at a rate of 0.5 to 0.8 cc. per minute, depending on the condition of the animal. When retching or vomiting occurred, the injection was stopped and the animal allowed a rest until the symptoms disappeared. No anesthetic was employed.

In the muscle trauma experiments, the dogs were deeply anesthetized with ether, and the thigh muscles struck a definite number of blows with a wooden mallet. The strength of the blows was just sufficient to severely bruise the muscles without producing bone fractures.

The animals used for intestinal manipulation were anesthetized with ether and, using strict asepsis, a three-inch slit made in the abdominal wall. A short segment of the small intestine was then lifted through the incision and gently stripped through the fingers, only a few inches of intestine being exposed at any time. The stripping was continued in this manner over the entire length of the small intestine for thirty minutes. The wound was then sutured and bandaged, and the dog allowed to recover from the anesthetic.

The arterial pressures were determined without anesthesia by the direct intra-arterial needle puncture method (13). The readings represent mean pressures. An initial reading was taken shortly before the experiment was begun, and all further pressures taken after recovery from the anesthetic. Since it has been shown (2) that, exclusive of the intraperitoneal glucose experiments, the serum Na, Cl, and K concentrations remain unchanged in the shock induced by these procedures, electrolyte studies were not made.

The dosage of D.C.A.[2] varied in the earlier experiments, but in the later work 15 mgm. were given in three doses of 5 mgm. each by intramuscular injection. The usual time interval for these injections was 18, 12 and 2

[2] We are indebted to the Ciba Pharmaceutical Products, Inc. for generous supplies of the desoxycorticosterone acetate (Percorten) used in these experiments.

hours before the experiment.   All cortical extract was withdrawn 36 hours before the experiment.   D.C.A. in oil can be used only for priming, i.e., as a prophylactic agent, since absorption after either subcutaneous or intramuscular injection is too slow to be of value once shock has developed.

I. *Intraperitoneal glucose injections.*   Six adrenalectomized dogs were primed with D.C.A. and subjected to intraperitoneal glucose injections. Typical protocols are shown in table 1.   None of these animals showed symptoms of circulatory failure or shock.   In contrast to the behavior of the D.C.A. treated dog is that of the nonprimed animal receiving the same amount of glucose (dogs 5, 6, table 1).   These animals exhibit circulatory collapse within 2 to 6 hours after the glucose administration, and will die within a few hours if left untreated.

II. *Epinephrine injections.*   A dose of 0.4 mgm. per kgm. body weight of epinephrine will produce fatal shock in the adrenalectomized animal maintained on cortical extract, while one of at least 0.7 mgm. per kgm. body weight is required to produce shock in either the intact dog or the adrenalectomized dog receiving priming doses of extract.   Epinephrine doses of 0.4 to 0.7 mgm. per kgm. were given seven adrenalectomized dogs, five being primed with D.C.A. and two untreated.   Representative data are given in table 2.   It is clear that the dog primed with D.C.A. is resistant to the amount of epinephrine which will produce fatal shock in the nontreated animal.   It is possible that it does not afford the same degree of protection as does cortical extract, for a D.C.A. primed animal receiving 0.7 mgm. per kgm. body weight (dog 4, table 2) did not show the expected spontaneous pressure rise.   Although this dosage was within the lower limits of the amount required to produce shock in the intact dog, cortical extract will usually protect the animal against even greater amounts of epinephrine.   Cortical extract was used to save the life of this animal.

III. *Muscle trauma.*   Typical protocols from the study of seven adrenalectomized dogs primed with D.C.A. and subjected to muscle trauma are given in table 3.   In striking contrast to the responses of dogs 1 to 4, which were primed previous to traumatization, and in which symptoms of shock did not develop, are those of dogs 5 and 6, not given treatment, in which death followed within nine hours.   The writers, in numerous experiments, have never observed an adrenalectomized dog receiving only maintenance doses of extract fail to develop circulatory collapse within a few hours after the same degree of trauma.   Animals primed with cortical extract likewise show no symptoms.

IV. *Intestinal stripping.*   Pertinent data from the study of eight adrenalectomized dogs after intestinal manipulation are given in table 4. Circulatory failure and shock occurred in all cases.   The D.C.A. priming apparently had no prophylactic action—a result in striking contrast to its protection against shock in the other experiments.   We have no ade-

quate explanation for its failure here. Negative results with D.C.A. after intestinal manipulation have also been reported by Selye and co-

TABLE 1

Action of desoxycorticosterone acetate on adrenalectomized dogs receiving intraperitoneal injections of isotonic glucose

DATE	TIME	BLOOD PRES-SURE	PULSE PER MINUTE	REMARKS

DATE	TIME	BLOOD PRES-SURE	PULSE PER MINUTE	REMARKS
	Dog 1. 10.2 kgm. Primed with D.C.A.			
		mm. Hg		
6/19	10:30 a.m.	98	72	Injected with glucose
	3:30 p.m.	73	140	No symptoms, active
	8:30 p.m.	68	140	No symptoms
6/20	4:00 a.m.	95	140	Normal, hungry
	Dog 2. 6.5 kgm. Primed with D.C.A.			
6/25	10:30 a.m.	88*	84	Injected with glucose
	3:30 p.m.	70	124	No symptoms
	8:30 p.m.	69	154	No symptoms, active
6/26	9:30 a.m.	89	92	Normal
	Dog 3. 9.1 kgm. Primed with D.C.A.			
7/1	10:00 a.m.	116	112	Injected with glucose
	3:00 p.m.	93	172	No symptoms
	11:00 p.m.	79	164	Active, no symptoms
7/2	10:00 a.m.	113	140	Normal
	Dog 4. 10.9 kgm. Primed with D.C.A.			
6/25	10:00 a.m.	104	96	Injected with glucose
	3:00 p.m.	75	140	No symptoms
	8:30 p.m.	72	152	No symptoms
6/26	9:30 a.m.	89	128	Appears normal. Experiment discontinued
	Dog 5. 9.3 kgm. No treatment, control			
5/16	10:30 a.m.	100	120	Injected with glucose
	4:30 p.m.	48	140	Very weak, walks with difficulty
	8:00 p.m.	38	140	Complete collapse. Revived with extract
	Dog 6. 8.9 kgm. No treatment, control			
5/18	11:00 a.m.	108	100	Injected with glucose
	7:30 p.m.	68	188	Depressed, inactive
	11:30 p.m.	46	140	In collapse. Revived with extract

* Animal had not received cortical extract for two days previous to priming with D.C.A.

workers (14) with intact rats, and by Weil and associates (15) using normal rabbits. They report that while cortical extract and corticosterone re-

duce the mortality from shock, D.C.A. was inactive and in some cases actually proved to be harmful.   That cortical extract will revive the animals used here is illustrated by dog 1, in which extract was used to save the animal's life after 20 mgm. D.C.A. failed to protect against shock.

TABLE 2

Protective action of priming doses of desoxycorticosterone acetate in preventing circulatory failure and shock following massive injections of epinephrine in adrenalectomized dogs

DATE	TIME	BLOOD PRES- SURE	PULSE PER MIN- UTE	REMARKS
				Dog 1. 9.4 kgm. Given 0.6 mgm. epinephrine/kgm. 15 mgm. D.C.A.
		mm. Hg		
10/8	10:00 a.m.	102	116	Injection started, finished at 10:50 a.m.
	12:00 m.	88	168	Depressed but *no* signs of weakness
	1:00 p.m.	94	152	Active, vigorous
	3:00 p.m.	104	152	Normal
				Dog 2. 10.4 kgm. Given 0.5 mgm. epinephrine/kgm. 15 mgm. D.C.A.
7/18	10:10 a.m.	92	80	Injection started, finished at 11:30 a.m.
	11:35 a.m.	53	160	Markedly depressed
	4:30 p.m.	80	120	Active, vigorous
7/19	10:30 a.m.	83*	76	Ate full ration, active
				Dog 3. 10.8 kgm. Given 0.5 mgm. epinephrine/kgm. 15 mgm. D.C.A.
7/18	2:20 p.m.	94	80	Injection started, finished at 3:30 p.m.
	3:40 p.m.	68	172	Depressed, inactive
	9:30 p.m.	89*	100	Active, vigorous
				Dog 4. 11.1 kgm. Given 0.7 mgm. epinephrine/kgm. 15 mgm. D.C.A.
10/14	9:40 a.m.	110	92	Injection started, finished at 10:50 a.m.
	11:00 a.m.	92	152	Depressed
	1:00 p.m.	72	160	Lethargic, depressed
	5:10 p.m.	58	168	Very weak. Given 3 cc./kgm. cortical extract
	11:00 p.m.	86*	140	Marked improvement, active, bright, ate food
				Dog 5. 9.8 kgm. Given 0.4 mgm. epinephrine/kgm. No treatment
6/5	9:30 a.m.	103	80	Injection started, finished at 10:20 a.m.
	3:20 p.m.	89	175	Inactive, depressed
	10:40 p.m.	50	180	Profound shock
	11:40 p.m.	36	200	Died 20 minutes later

* Experiment discontinued.

Four of the eight dogs exhibited unusual symptoms which were quite alien to those of shock.   There seemed a correlation between the amount of D.C.A. given, the time allowed for absorption, and the appearance of

TABLE 3

Effectiveness of priming doses of desoxycorticosterone acetate in preventing circulatory failure and shock following muscle trauma in adrenalectomized dogs

DATE	TIME	BLOOD PRES-SURE	PULSE PER MINUTE	REMARKS
				Dog 1. 12.6 kgm. 30 mgm. D.C.A.
4/22	4:00 p.m.	*mm. Hg* 106	120	Primed with 10 mgm. D.C.A. at 10:30 a.m., 1:30 p.m. and 4:30 p.m. 80 blows to right hind leg muscles at 4:30 p.m.
	8:30 p.m.	99	120	
	11:30 p.m.	92	147	No symptoms. Leg greatly swollen. Dog active, bright
4/23	10:30 a.m.	92	130	Hops about in cage. Ate full ration
	4:30 p.m.	96	120	Normal
				Dog 2. 10.7 kgm. 15 mgm. D.C.A.
7/10				Primed with 5 mgm. D.C.A. at 5 p.m., 11 p.m. and at 8 a.m. on 7/11
7/11	10:00 a.m.	99	60	Given 100 blows to right hind leg muscles
	4:30 p.m.	89	128	No symptoms. Leg swollen, hops about in cage. Bright
	11:30 p.m.	94	160	Active and vigorous. Ate full ration eagerly
7/12	8:30 a.m.	96	88	Leg still swollen. Active, vigorous
				Dog 3. 11.1 kgm. 15 mgm. D.C.A.
7/11				Primed with 5 mgm. D.C.A. at 5 p.m., 11 p.m. and at 8 a.m. on 7/12
7/12	10:00 a.m.	86*	80	Given 95 blows to right hind leg muscles
	3:30 p.m.	72	142	Leg swollen. No symptoms, dog quiet
	11:30 p.m.	64	140	Active, ate full ration eagerly
7/13	9:30 a.m.	72	140	Leg still swollen. Active, alert
7/14	9:30 a.m.	86	90	Normal
				Dog 4. 11.4 kgm. 5 mgm. D.C.A.
7/14				Primed with 5 mgm. D.C.A. at 11 p.m.
7/15	9:30 a.m.	108	120	Given 110 blows to right hind leg muscle
	2:30 p.m.	108	152	No symptoms. Leg swollen
	11:30 p.m.	103	160	Ate full ration eagerly
7/16	9:30 a.m.	105	120	Normal
				Dog 5. 10.5 kgm. No treatment
8/30	12:30 p.m.	101	85	Given 97 blows to right hind leg muscles
	7:00 p.m.	83	174	Depressed, dull. Leg greatly swollen
	9:30 p.m.	35	173	In collapse. Died at 10:15 p.m.
				Dog 6. 14.0 kgm. No treatment
8/30	10:00 a.m.	100	70	Given 100 blows to right hind leg muscles
	12:15 p.m.	72	98	Depressed
	2:40 p.m.	56	140	Weak, symptoms of shock
	4:15 p.m.	42	176	Prostrate
	7:10 p.m.	36	182	Died at 8:00 p.m.

* Animal had low pressure before adrenals removed.

TABLE 4

Non-effectiveness of priming doses of desoxycorticosterone acetate in preventing circulatory failure and shock following intestinal manipulation in adrenalectomized dogs

DATE	TIME	BLOOD PRES- SURE	PULSE PER MINUTE	REMARKS
				Dog 1. 10.0 kgm. 30 mgm. D.C.A.
		mm. Hg		
6/2				Given 10 mgm. D.C.A. at 9:30 a.m. and at 9:30 a.m. and 12:15 p.m. on 6/3
6/3	11:45 a.m.	104	100	Given 30' intestinal stripping
	3:15 p.m.	52	130	Prostrate
	8:15 p.m.	34	140	Still in collapse. Unusual symptoms*
	11:00 p.m.	36	140	Given 30 cc. cortical extract
6/4	10:00 a.m.	70	140	Bright and alert. Ate full ration. 30 cc. extract
6/5	10:00 a.m.	98	100	Normal
				Dog 2. 12.0 kgm. 20 mgm. D.C.A.
6/2				Given 10 mgm. D.C.A. at 9:30 a.m. on 6/2 and 6/3
6/3	2:15 p.m.	106	90	Given 30' intestinal stripping
	4:45 p.m.	102	120	Active, no symptoms
	7:45 p.m.	68	160	Depressed, unusual symptoms*
	10:45 p.m.	33		Complete collapse. Died at 11 p.m.
				Dog 3. 10.1 kgm. 15 mgm. D.C.A.
7/1				Given 5 mgm. D.C.A. at 5 p.m., 11 p.m. and 8 a.m. on 7/2
7/2	9:45 a.m.	105	80	Given 30' intestinal stripping
	1:30 p.m.	109	120	Active, no symptoms
	4:30 p.m.	85	160	No symptoms
	9:00 p.m.	68	160	Depressed, lethargic
	11:00 p.m.	56	168	Bloody diarrhea, in collapse, given 30 cc. cortical extract
7/3	7:30 a.m.	48	168	Little improvement. Given 30 cc. extract
	3:30 p.m.	73	148	Bright, active, ate food
7/4	9:30 a.m.	100	90	Normal
				Dog 4. 6.6 kgm. 15 mgm. D.C.A.
6/30				Given 5 mgm. D.C.A. at 5 p.m., 11 p.m. and 8 a.m. on 7/1
7/1	10:15 a.m.	99	74	Given 30' intestinal stripping
	12:45 p.m.	87	68	Bloody stools, depressed
	3:45 p.m.	45	180	Very weak
	4:30 p.m.	34	182	Collapse. Died within an hour

* See text.

TABLE 4—*Concluded*

DATE	TIME	BLOOD PRES- SURE	PULSE PER MINUTE	REMARKS
Dog 5. 10.4 kgm. No treatment				
		mm. Hg		
5/16	10:30 a.m.	110	132	Given 30′ intestinal stripping
	12:30 p.m.	82	200	Depressed
	4:40 p.m.	42	180	Collapse. Died at 6:45 p.m.
Dog 6. 11.1 kgm. No treatment				
5/16	10:30 a.m.	110	132	Given 30′ intestinal stripping
	1:15 p.m.	85	152	No symptoms
	4:15 p.m.	45	152	Very weak, shock
	8:15 p.m.	32		Died on table

the symptoms. They have not been observed in any of the experimental procedures other than intestinal stripping. Within a few hours after the intestines were stripped, an excitability with muscular twitching developed. Eventually convulsive seizures ensued, with rigid extension of the forelegs, generalized muscle tremors, and opisthotonus. The convulsive state was followed by extreme muscular weakness with flaccidity of the neck muscles. The dog was in semi-conscious state throughout. These symptoms resemble those reported by Kuhlmann and associates (16) in intact dogs after excessively large doses of D.C.A. had been given for a relatively long period of time. These authors ascribed the symptoms to an abnormally low serum potassium level.[3]

DISCUSSION. The writers have advocated for some years that an important function of the adrenal cortex lies in its "pressor" action at the vascular periphery. This is not to imply that the hormone acts directly to raise the blood pressure, as does a true pressor drug, but only that the secretion of the adrenal cortex is necessary in some way for the maintenance of the normal ability of the periphery to cope with a vascular strain.

One of the cardinal features of adrenal insufficiency in the dog is the slow but steady decline in arterial pressure, a fall which appears before the blood picture has been appreciably altered. By the time the animal is showing marked symptoms of insufficiency, the pressure is at "shock" levels. Even when the dog is maintained in excellent health by adequate doses of extract, a sudden stress on the vascular periphery (e.g., a prolonged vasoconstriction) leads to a slow but progressive fall in blood

[3] Further experimentation has shown that corticosterone adequately protects against circulatory failure induced by intestinal stripping. D.C.A. affords no protection against circulatory collapse resulting from one other type of trauma, while cortical extract is highly effective.

pressure and eventual circulatory collapse within 6 to 10 hours. We have observed no instance when the pressure could be spontaneously raised and maintained after this progressive decline had started. Adequate amounts of cortical extract will interrupt the fall, and restore the circulation to normal, even though the pressure has reached "shock" levels. If the adrenalectomized animal has been previously primed with extract, the resistance to peripheral vascular strain is as marked as in the intact dog. It should be borne in mind that the only change manifest in this experimentally induced shock is the low blood pressure, for serum electrolyte concentrations, blood glucose, etc., remain normal. Hence the normal picture of adrenal insufficiency has not been reproduced, the procedures used sufficing only to throw into exaggerated light the inability of the adrenalectomized dog to make peripheral vascular capacity adjustments in the absence of hormone and the restoration of this ability when hormone is given. The evidence would indicate a fundamental role of the adrenal cortical hormone in maintenance of the functional integrity of some part of the peripheral vascular apparatus.

Since D.C.A. will protect the adrenalectomized animal against shock after muscle trauma, epinephrine injections, and intraperitoneal glucose injections, it, too, presumably has an action on the peripheral vasculature. It may not afford a protection equal to that of cortical extract. The failure to protect against shock after intestinal stripping indicates that at least in this particular experiment D.C.A. is not identical with cortical extract.

SUMMARY

1. Desoxycorticosterone acetate successfully protected the adrenalectomized dog against circulatory collapse and shock following the intraperitoneal injection of an isotonic glucose solution, the injection of large amounts of epinephrine, and trauma to muscle masses.

2. In contrast, no evidence of an effective protection was found against the shock following intestinal stripping.

3. When large doses of desoxycorticosterone acetate were used in the intestinal stripping experiments, unusual symptoms appeared which the writers have never before observed accompanying this type of shock.

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THE INFLUENCE OF VITAMIN E-DEFICIENCY ON THE ENDO-CRINE GLANDS OF RATS, PARTICULARLY ON THE GONADOTROPIC HORMONE CONTENT OF THE PITUITARY GLAND[1]

CLYDE BIDDULPH AND ROLAND K. MEYER

From the Department of Zoölogy, University of Wisconsin, Madison

Studies concerning the influence of vitamin E-deficiency of rats upon the gonadotropic hormone content of the pituitary gland are conflicting. It has been reported that there is an increase in the male (1, 2), and no effect (1), an increase (3), and a decrease (4, 2) in the female. The present study was undertaken to reinvestigate the problem upon a large number of assay animals. It was also desired to determine whether each component of the gonadotropic complex (follicle stimulating and luteinizing hormone) was influenced quantitatively in instances where changes in the gonadotropic hormone content of the pituitary gland were produced. Complete autopsy data on all the endocrine glands of large groups of normal, vitamin E-deficient, and castrate male and female rats were taken. The gland weights of these rats are included in this report.

MATERIALS AND METHODS. The diet used to produce the vitamin E-deficiency was the standard E-low diet[2] (diet 427) of Evans, et al. (5, 6). Rats from our own colony, which are of the Sprague-Dawley strain, were used in all the experiments. They were placed on the E-deficient diet at one month of age and maintained on that diet until autopsy, which was performed at 4, 7, 13 and 16 months of age. The animals received the diet ad libitum. Groups of rats were gonadectomized at one month of age and kept until 4, 7 and 13 months of age before autopsy was performed. Normal animals of the same age were sacrificed with each group, so that it is possible to compare data from normal, E-deficient and castrate males at the ages indicated. Comparisons of all three types of females were made only at 7 months of age. The normal and castrate animals were fed with Wayne dog chow.

The animals were killed with ether and then autopsied. The pituitary gland was immediately removed, weighed and crushed between two glass plates. The pituitary tissue was allowed to dry at room temperature for 3 to 5 days, after which it was removed from the plates with a razor blade, weighed and stored in individual vials in a desiccator. Data will be presented to show that there is no appreciable loss of potency of glands stored in this manner for as long as 16 months.

Subsequent to the removal of the pituitary gland, the remaining endocrine glands were removed and weighed to the nearest milligram.

The pituitary glands of the different groups were assayed after all the glands had been collected. At this time they had been stored for one-half to 15 months. Female rats of the Sprague-Dawley strain, weighing from 34 to 37 grams, were brought into the laboratory at 21 days of age. On the 25th day of life they were hypophysectomized and the following day injections of the pituitary material were begun. The pituitary powder was easily made into a fine suspension by flushing the material back and forth into a syringe through a 23 gauge hypodermic needle. The injections were made subcutaneously twice daily for $4\frac{1}{2}$ days with autopsy on the morning of the sixth day, at which time the ovaries, adrenals and thyroids were removed, examined and weighed. Three animals were injected with each dosage of pituitary powder, thus a total of 162 hypophysectomized animals were used in the assays.

RESULTS AND DISCUSSION. *Endocrine gland weights of male rats.* An analysis of the autopsy data of the 311 male rats included in table 1 shows the following:

1. The testes of the E-deficient males weighed less than one-half as much as the testes of normal males. The atrophy was as marked after 3 months of E-deficiency as after 12 or 15 months, when judged on a weight basis. Histological sections, however, showed that the degeneration of the seminiferous epithelium was not complete after 3 months, but was complete after 6, 12, and 15 months. Supplementing the diet with wheat germ oil[3] (0.2 cc. per day for the first 3 months and 0.3 cc. per day for the following 3 months) maintained normal testis weight and a normal quantity of sperm in the seminiferous tubules. There was no detectable difference in the amount of interstitial tissue in the testes of the E-deficient and normal rats at any age.

2. The weight of the seminal vesicles and prostate of the E-deficient males, when analyzed statistically, was significantly greater than that of the corresponding glands of normal rats at 4 months of age. The weight difference of these organs at 7 and 13 months was not statistically significant. Supplementing the diet with wheat germ oil gave accessory glands

that were significantly heavier than those of both normal and E-deficient animals. This response may indicate that wheat germ oil possesses androgenic or gonadotropic properties. Verzar (7) claimed that injections of wheat germ oil induced precocious development of the uterus of rats, but Diakov and Krizenecky (8) were unable to confirm this.

TABLE 1

Autopsy data of normal, vitamin E-deficient and castrate male rats

TREATMENT	AGE AT AUTOPSY	NO. RATS	BODY WT.*	GONADS	SEM. VESC.	PROS-TATE	AD-RENALS	THY-MUS	THY-ROID	PITUIT.
	mo.									
Normal	4	26	245 ±4.88	3021 ±50.78	654 ±30.80	602 ±26.85	36 ±1.65	340 ±18.29	12 ±0.32	6.9 ±0.14
E-def. 3 mo.	4	37	310 ±6.17	1246 ±25.27	861 ±22.15	731 ±19.98	43 ±0.99	361 ±10.00	15 ±0.36	8.4 ±0.17
Castr. 3 mo.	4	33	256 ±4.28		10 ±0.31	18 ±0.49	47 ±1.19	361 ±16.20	13 ±0.37	12.3 ±0.24
Normal	7	31	326 ±7.80	2861 ±83.20	688 ±45.50	683 ±30.80	34 ±0.80	183 ±10.80	15 ±0.44	7.3 ±0.01
E-def. 6 mo.	7	30	313 ±7.05	1240 ±32.54	780 ±47.18	727 ±48.55	41 ±1.08	300 ±12.70	32 ±0.85	9.0 ±0.25
E-def. + wht. germ oil** 6 mo.	7	32	392 ±6.72	2977 ±50.56	993 ±43.31	941 ±31.55	47 ±1.46	279 ±8.77	20 ±0.50	9.1 ±0.20
Castr. 6 mo.	7	36	324 ±7.41		37 ±2.03	44 ±2.03	45 ±1.46	283 ±13.60	17 ±0.52	14.8 ±0.36
Normal	13	23	370 ±9.83	3060 ±58.95	973 ±57.11	1009 ±38.95	41 ±1.50	202 ±16.28	17 ±0.64	9.1 ±0.27
E-def. 12 mo.	13	28	307 ±8.52	1222 ±52.53	1067 ±61.02	902 ±45.11	56 ±2.05	174 ±10.03	25 ±0.65	9.4 ±0.25
Castr. 12 mo.	13	28	340 ±9.79		15 ±0.69	19 ±0.68	51 ±1.34	179 ±42.59	17 ±0.70	15.7 ±0.52
E-def. 15 mo.	16	7	288 ±19.73	1338 ±125.83	682 ±125.07	709 ±108.71	65 ±2.30	137 ±24.58	26 ±1.85	10.8 ±0.56

* The body weight is in grams; all other weights are in milligrams. The standard error of the mean of each weight is also given.

** Each rat received 0.2 cc. of wheat germ oil per day from a syringe for the first three months and 0.3 cc. per day for the following three months.

The weight of the seminal vesicles and prostate after 15 months of E-deficiency was 36 and 21 per cent respectively, less than the weight of these glands after 12 months. This decrease was correlated with the condition of the animals at this age, for the muscular dystrophy and weakness characteristic of long periods of E-deficiency (9) were very marked. It is doubtful, however, whether this atrophy can be attributed solely,

if at all, to the E-deficiency, for it has been reported that inanition induces an atrophy of the accessory glands (10). Kudrjaschov (11) has claimed that E-deficiency *per se* produces atrophic accessory glands.

3. The adrenal glands of the E-deficient males were intermediate in weight between the adrenals of normals and castrates at all ages except 13 months. At the latter age they were heavier than those of both normals and castrates, and after 15 months they had increased further in weight. The muscular dystrophy and weakness were clearly more pronounced at the latter age, therefore it seems that this rapid increase in weight might possibly be attributed to the alarm reaction which has been described by Selye (12, 13).

Hatai (14), Anderson and Kennedy (15) and Winter and Emery (16) have all reported that gonadectomy induced hypertrophy of the adrenal in male rats, but atrophy in the female. The results of the present report confirm these observations. (See also table 2.)

The width of the cortex and medulla was measured from histological sections with an ocular micrometer to determine which part of the gland was responsible for the weight increase. In the castrate and E-deficient animals, the width of the fasiculate and reticular layers of the cortex was increased, the greatest increase occurring in the castrates. The glomerular layer was unchanged in width. Both groups showed the greatest increase over normals at 13 months of age. The data of Drummond et al. (2) do not show any increase in adrenal weight of male E-deficient rats, whereas those of Evans et al. (6) show a definite increase. The present data agree with those of Evans and co-workers.

4. Involution of the thymus did not occur as early in the E-deficient and castrate male rats as in the normals. Involution in the latter had taken place at 7 months of age, but it did not occur in the E-deficient animals until 13 months of age. It is possible that the thymus involuted earlier than 13 months, but data are not available because animals were not autopsied in the interval between 7 and 13 months.

Since it is generally accepted that androgen and estrogen hasten the involution of the thymus, the results of the E-deficient animals are difficult to explain. Apparently more male hormone was being produced in these animals, as was evidenced by the increase in the accessory gland weights, but regardless of this increase, thymus involution did not occur as soon as in the normal animals.

5. The thyroids of the E-deficient males were significantly heavier than the thyroids of normals and castrates at all ages. The greatest enlargement occurred in the 6 month E-deficient animals. Supplementing the diet with wheat germ oil gave thyroids that were only slightly heavier than those of normals. The addition of iodine to the E-deficient diet main-

tained the thyroid weight at the normal level and also maintained normal thyroid histology. These data will be presented in a subsequent report.

6. Vitamin E-deficiency in the male produced an increase, which was statistically significant, in the weight of the pituitary gland of the groups sacrificed at 4 and 7 months of age, but there was not a significant increase in the group sacrificed at 13 months. The percentage increase was 21, 23 and 3 per cent at 4, 7 and 13 months respectively. These data indicate that there was an influence on pituitary weight after 3 and 6 months but not after 12 months of E-deficiency. The pituitary glands of animals that received a supplement of wheat germ oil were 24 per cent heavier than those of normals. This increase, which is significant statistically, is approximately the same as that of E-deficient animals of the same age. The germinal epithelium was normal in these animals regardless of the increase in size of the pituitary gland. The reason for this weight increase is not known. The data of Evans et al. (6), from a much smaller number of animals, likewise show an increase in the weight of the pituitary gland of E-deficient and E-deficient animals given a supplement of wheat germ oil. However, the increase was considered non-significant by these workers.

Castration significantly increased the weight of the pituitary gland over that of normal animals at each age, the percentage increase being 78, 102, and 72 per cent at 4, 7, and 13 months respectively.

Endocrine gland weights of female rats. The autopsy data of the 170 female rats reported in table 2 are summarized as follows:

1. Vitamin E-deficiency did not significantly influence the weight of the ovary in any of the age groups studied. The cyclic function of the ovary was maintained for at least 12 months of E-deficiency, for it was found that females at this age had normal estrous cycles. Evans et al. (17) have previously reported that the estrous cycles of E-deficient females were normal.

2. The adrenal weight of the 6 month ovariectomized females was significantly less than the adrenal weight of normal and E-deficient animals of the same age. Hatai (14), Anderson and Kennedy (15) and Winter and Emery (16) have reported the same observation in ovariectomized female rats.

The difference between the adrenal weights of the E-deficient and normal animals was not significant at any age. Blumenfeld (18) reported that vitamin E-deficiency produced an atrophy of the medulla but a hypertrophy of the cortex in female rats. However, the absolute weight of the gland was not significantly different from that of normals. In the present study there was no alteration in absolute weight of the adrenal or in the width of the cortex or medulla.

3. The difference between the thymus weights of the E-deficient and normal females was not significant at any age at which comparisons are possible. The difference between the thymus weight of the normal and ovariectomized animals is significant, which indicates that ovariectomy delays thymus involution.

4. The thyroid hypertrophy obtained in the male E-deficient animals was not evidenced in the females, for there was no significant difference in thyroid weights of any of the groups.

TABLE 2

Autopsy data of normal, vitamin E-deficient and ovariectomized female rats

TREATMENT	AGE AT AUTOPSY	NO. RATS	BODY WT.*	GONADS	ADRENALS	THYMUS	THYROID	PITUI-TARY
	mo.							
E-def. 3 mo.	4	34	223 ±3.48	47 ±1.83	49 ±1.20	306 ±10.48	14 ±0.30	9.9 ±0.22
Normal	7	33	237 ±3.28	48 ±1.34	55 ±1.19	199 ±9.75	17 ±0.69	12.4 ±0.36
E-def. 6 mo.	7	36	230 ±4.59	43 ±1.62	58 ±1.54	222 ±8.36	15 ±0.42	12.7 ±0.36
Ovariectomized 6 mo.	7	31	260 ±4.47		45 ±1.57	234 ±7.68	15 ±1.10	12.3 ±0.28
Normal	13	6	245 ±6.48	33 ±3.09	66 ±5.10	138 ±7.62	15 ±1.08	13.9 ±1.13
E-def. 12 mo.	13	30	221 ±3.90	38 ±2.12	68 ±1.67	168 ±9.20	17 ±0.42	14.8 ±0.40

* The body weight is in grams; all other weights are in milligrams. The standard error of the mean of each weight is also given.

5. Vitamin E-deficiency did not alter the weight of the pituitary gland from that of normal female rats. This has been reported previously by Nelson (1) and Stein (19). Ovariectomy likewise did not influence the weight of the pituitary gland. This is in agreement with the reports of the majority of investigators, which have been summarized by Lawson et al. (20).

Assay of pituitary glands for gonadotropic hormone. The results of the pituitary assays of the various groups of rats are presented in tables 3 and 4. The average adrenal and thyroid weights of the hypophysec-tomized test animals indicate that in general the larger quantities of pituitary powder gave heavier adrenals and thyroids than the smaller

TABLE 3

Assay of male rat pituitary glands

DONOR				HYPOPHYSECT. RECIPIENT*			
Treatment	Age	Time stored	Amount injected	Av. ovar. wt.**	No. rats with corpora lutea	Av. adren. wt.**	Av. thyr. wt.**
	mo.	mo.	mgm.	mgm.			
Normal...............	4	7	1	10	0	8.7	5.0
			2	56	0	8.0	4.5
			4	69	1	7.5	5.8
			6	65	0	9.0	3.9
E-def. 3 mo...............	4	6	1	14	0	9.5	6.7
			2	46	1	8.6	4.5
			4	76	2	9.1	4.5
			6	94	3	9.0	4.8
Castrate 3 mo.............	4	5	1	14	1	7.5	5.7
			2	90	3	7.5	3.3
			4	78	3	8.9	4.6
			6	133	3	8.3	4.7
Normal...............	7	11	1	5	0	6.1	3.1
			2	20	0	8.2	5.9
			4	35	1	8.1	5.9
			6	53	0	8.0	5.8
E-def. 6 mo...............	7	15	1	7	0	7.4	3.9
			4	49	0	7.3	5.5
			6	47	3	8.8	5.8
E-def. + wht. germ oil 6 mo...	7	6	1	8	0	8.3	4.0
			2	19	0	8.1	5.0
			4	41	0	7.6	5.4
			6	46	0	9.0	6.0
Castrate 6 mo.............	7	8	1	27	1	6.3	3.9
			2	76	3	8.3	4.9
			4	90	3	8.2	4.8
			6	83	3	9.0	6.0
Normal...............	13	8	1	7	0	6.8	3.9
			2	22	0	7.4	4.2
			4	49	0	8.3	5.3
			6	65	2	9.0	5.0
E-def. 12 mo...............	13	8	1	8	0	6.8	4.2
			2	21	0	6.8	4.5
			4	49	1	7.4	4.9
			6	60	3	7.7	4.3
Castrate 12 mo.............	13	1	1	22	1	7.7	4.7
			2	69	3	8.0	3.8
			4	81	3	8.0	5.0
			6	78	3	8.8	4.9

* The average ovarian, adrenal and thyroid weight of 5 operated controls was 4, 7, and 3.7 mgm. respectively.

** Average of 3 animals.

quantities. There is no indication that the thyrotropic or adrenotropic hormone content of the E-deficient pituitaries was increased. From the increase in thyroid weight, especially of the 6 month E-deficient males, it might be expected that more thyrotropic hormone was produced, but the data do not show any increase. If a test animal were used which was more sensitive to thyrotropic hormone, an increase might be detected.

TABLE 4

Assay of female rat pituitary glands

DONOR				HYPOPHYSECT. RECIPIENT[*]			
Treatment	Age	Time stored	Amount injected	Av. ovar. wt.[**]	No. rats with corpora lutea	Av. adren. wt.[**]	Av. thyr. wt.[**]
	mo.	mo.	mgm.	mgm.			
E-def. 3 mo................	4	6	1	7	0	9.0	3.0
			4	9	0	7.8	4.0
			6	11	0	8.0	5.0
Normal.....................	7	7	1	5	0	7.3	3.6
			4	7	0	7.5	4.6
			6	7	0	8.7	4.7
E-def. 6 mo................	7	3	1	5	0	7.6	3.6
			4	7	0	7.6	4.9
			6	8	0	8.0	4.4
Ovariectomized 6 mo........	7	5	1	16	0	6.7	3.9
			4	69	3	7.8	4.7
			6	93	3	9.0	6.0
E-def. 12 mo...............	13	½	1	7	0	7.5	5.0
			4	6	0	8.3	4.2
			6	6	0	7.6	4.6

[*] The average ovarian, adrenal and thyroid weight of 5 operated controls was 4, 7 and 3.7 mgm. respectively.

[**] Average of 3 animals.

The ovarian weights obtained from the assay of male pituitary glands were analyzed statistically by the technique of analysis of variance (21) to determine whether the apparent differences between ovarian weights were significant. This type of analysis allows the probabilities of chance differences of the size found between normal and E-deficient or normal and castrate animals for each dosage or age, to be combined into a single probability for testing the significance of the average difference over all groups.

The data in table 5 show that there are no highly significant[4] differences between the response from any particular dosage of E-deficient pituitary over all ages and the response from the same dosage of normal pituitary. The difference at 1 mgm., though on the border-line of significance statistically, is not considered significant biologically because of the small amount of stimulation above the control level of non-injected hypophysectomized controls. On the other hand, all of the differences between the response from any particular dosage of castrate pituitary over all ages, and the response from the same dosage of normal pituitary, are significant with the exception of the difference at 4 months. The 4 month castrate group, however, was consistently higher than the normal group

TABLE 5

Probability of the differences between ovarian weights occurring by chance in the assay of male rat pituitary glands

PROBABILITY COMBINATIONS	GROUPS COMPARED	
	Normal and E-deficient	Normal and castrate
	Probability	*Probability*
1. All ages at each dosage (i.e., 1 mgm. at 4, 7, 13 mo. etc.):		
1 mgm..	0.04	0.002
2 mgm..	0.37	0.0002
4 mgm..	0.33	0.03
6 mgm..	0.49	0.01
2. All dosages at each age (i.e., 1, 2, 4, 6 mgm. at 4 mo. etc.):		
4 mo...	0.51	0.30
7 mo...	0.27	0.004
13 mo..	0.60	0.002
3. All ages at all dosages (i.e., 1, 2, 4, 6 mgm. at 4, 7, 13 mo.)	0.26	<0.001

at all dosages, thereby agreeing with all other comparisons in the direction of the difference between castrate and normal.

The foregoing analysis shows that there is no significant difference in the ovarian weights obtained from the assay of normal and E-deficient male pituitary glands on a per unit weight basis, regardless of comparisons of the different combinations that were made. If, however, our assays were performed on a whole gland basis it would seem that the potency of the male glands would probably be increased after 3 and 6 months of E-deficiency. The percentage increase in total weight of the pituitary gland at these ages was 21 and 23 per cent, so if 21 to 23 per cent more pituitary powder were injected, it is probable that there would be an increase in the ovarian weights obtained. Whether such an increase would

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[4] A probability >0.05 was considered to be statistically nonsignificant, 0.01 to 0.05 significant, and <0.01 highly significant or indicative of a real difference.

be statistically significant cannot be predicted. No increase in ovarian weight would be expected after 12 months of E-deficiency because there was no increase in the total weight of the pituitary gland at this age. Therefore, upon a per unit weight basis of pituitary material the present results do not confirm those of Nelson (1) and Drummond (2), but if the assays were performed on a per gland basis, it is probable that they would after 3 and 6 months of E-deficiency, but not after 12 months.

The assays of the female pituitary glands (table 4) do not show any difference in the ovarian response of the E-deficient and normal glands. The low level of response from the highest dosage of pituitary makes conclusions regarding the relative potency impossible. The pituitaries from the females that were ovariectomized for 6 months gave a substantial increase in ovarian weight, therefore it may be concluded that these glands are more potent than either the E-deficient or normal pituitaries.

To ascertain whether there was any deterioration of pituitary powder prepared and stored in the manner described above, a pooled sample of dried rat pituitary powder was assayed on normal 21 day-old female rats at intervals during a period of 16 months. Each rat in each assay received 2 mgm. of the dry powder. The average ovarian response obtained after storage for 4 months was 64 mgm. (3 rats); after 7 months, 64 mgm. (7 rats); and after 16 months, 58 mgm. (6 rats). These results indicate that there was no appreciable loss in potency when dried pituitary powder was stored for as long as 16 months. In view of these data it seems unlikely that there was any loss in potency of the pituitary powder from the normal, E-deficient and castrate rats reported above, for the longest period of storage of pituitary powder from these groups was 15 months.

The number of rats in each group having corpora lutea in their ovaries following the injection of the pituitary powder was taken as the criterion of the amount of luteinizing hormone in the pituitary material. The data in tables 3 and 4 indicate that castrate pituitary glands contain more luteinizing hormone (LH) than E-deficient glands, and that E-deficient male pituitaries contain more than those of normals. The pituitary glands from ovariectomized females were the only ones of this sex that formed corpora lutea in the ovaries of the test animals. Emanuel (22), Evans et al. (23), and more recently Hellbaum and Greep (24) have reported that luteinizing hormone is increased in amount in the pituitary gland of castrate animals.

The increased luteinizing capacity of the pituitary glands of the E-deficient males is correlated with the following anatomical alterations in these animals: 1, degeneration of the germinal epithelium; 2, increase in size of the accessories after 3 and 6 months, but no increase after 12 months of E-deficiency; 3, increase in weight of the pituitary gland after

3 and 6 months, but no increase after 12 months of E-deficiency, and 4, increase in size and number of basophils in the anterior lobe of the pituitary gland after 6 months of E-deficiency.   There is no indication from the data that the follicle stimulating hormone (FSH) content of these pituitary glands was also increased although it may have been.   However, the increase in size of the accessory glands of the 3 and 6 month E-deficient animals and the increase in amount of luteinization obtained in the assay of their pituitary glands, are definite indications that the LH content of the pituitary gland was increased.   Although there was some evidence of an increase in luteinizing activity of the 12 month E-deficient male pituitaries, there was no increase in the weight of their accessory glands when compared with control animals.

When the data from the assay of the castrate male pituitary glands were plotted, it was found that the ovarian weights obtained as a result of injecting the pituitaries from the 3 months castrates, did not reach a plateau on the highest dosage of pituitary powder (6 mgm.).   The curves from the assay of the 6 and 12 month castrate pituitaries were practically identical, for each attained a maximum at 4 mgm. of pituitary powder.   There was no further increase in the height of the curve with 6 mgm. of pituitary material, but rather a slight decrease.   This difference in response between the 3 month castrates on the one hand and the 6 and 12 month castrates on the other, indicates either that the pituitary glands of young adult castrate rats are more potent than those of older animals, or that the rate of increase in activity following castration is most rapid at a young age, after which a decline in potency occurs.   Whether the increase in potency of the castrate pituitary glands represents an increase in FSH as well as LH (for it is probable that more luteinization and hence a greater ovarian weight would be obtained merely by increasing the quantity of LH), or whether both components increase simultaneously, is not indicated by the present data.   Careful quantitative studies must be made with hypophysectomized female rats, using varying ratios of follicle stimulator to luteinizer, before this can be answered.

From the fact that the gonadotropic potency of the E-deficient pituitaries did not increase following the destruction of the germinal epithelium, the question arises as to the relative importance of the germinal epithelium and the interstitial tissue of the testes in the reciprocal gonad-pituitary interrelationship.   It is evident from the data that removal of both the germinal epithelium and interstitial tissue by castration, greatly increased the gonad stimulating activity of the pituitary gland.   Removal of the germinal epithelium alone by vitamin E-deficiency did not increase the gonadotropic activity; therefore it would seem that the interstitial tissue is more important in the control of the pituitary gland.   Witschi et al. (25) have reported that there was more gonadotropic hormone

in the blood stream of x-ray sterilized males in parabiosis with normal females, than in normal animals. However, no attempt was made to assay the pituitary glands of the parabiotics, so their data cannot be directly compared with those presented in this report because they determined the amount of gonadotropic hormone in the bloodstream and not in the pituitary gland.

We are greatly indebted to Dr. A. B. Chapman of the Department of Genetics for assistance in the statistical analysis of the data obtained from the assay of the pituitary glands.

SUMMARY

1. Endocrine gland weights of 234 vitamin E-deficient male and female rats, and 247 gonadectomized and normal male and female rats which served as controls for the E-deficient animals, are presented. An analysis of the data shows the following effects of vitamin E-deficiency:

a. Degeneration of the germinal epithelium with a consequent decrease in testis weight to a level which was less than one-half that of the testes of normal animals. There was no effect on the weight or on the cyclic function of the ovary.

b. An increase in accessory gland weight after a short period of deficiency (3 mos.), but a decrease after longer periods (15 mos.). Supplementing the diet with wheat germ oil produced the heaviest accessory glands obtained in any of the animals.

c. The adrenals of the males increased in weight due to hypertrophy of the cortex, whereas there was no influence on the weight of the adrenals of the female.

d. Involution of the thymus was delayed in the male but not in the female.

e. The thyroids of the males increased as much as 100 per cent in weight, whereas those of the females were not affected. The increase in weight of the male thyroids was largely prevented by supplementing the diet with wheat germ oil, and completely prevented by supplementing with iodine.

f. The pituitaries of the males increased in weight after 3 and 6 months of E-deficiency. There was no increase after 12 months of deficiency. The basophils increased in size and number after 6 months of deficiency. The weight of the female E-deficient pituitary gland was not different from that of normals.

2. Statistical analysis of the ovarian weights obtained following the injection of E-deficient male and normal male pituitary powder into immature hypophysectomized female rats, failed to show a significant difference in the gonadotropic potency. Pituitary glands from castrate rats

produced a significant increase in ovarian weight over that produced by normal glands. Pituitaries from E-deficient females produced the same ovarian response as those from normal females.

3. There was an increase in the luteinizing hormone content of the pituitary glands of vitamin E-deficient males when compared with normals. This increase was not as great as that obtained with those of castrate males.

4. Data are presented which show that there is no appreciable loss in potency of dried rat pituitary glands stored for as long as 16 months.

5. The bearing of the results upon the general problem of the gonadal-pituitary interrelationship is discussed.

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A CORRELATION OF THE pH OF ARTERIAL BLOOD AND URINE AS AFFECTED BY CHANGES IN PULMONARY VENTILATION[1, 2, 3]

CHARLES R. BRASSFIELD AND VIVIAN G. BEHRMANN

From the Department of Physiology, University of Michigan, Ann Arbor

This investigation was undertaken primarily to study simultaneously the pH changes in arterial blood and urine as measured continuously with glass electrodes and produced by variations in composition of the respiratory gases. Urine analyses were made for ammonia, phosphates and chlorides for information relative to the concentration of substances concerned in the urine pH changes.

METHODS. Ten to fifteen kilogram dogs anesthetized with sodium pentobarbital were used in these experiments. Ureteral catheters (French no. 4 or no. 5) were shortened and used as cannulae for the ureters exposed by a retro-peritoneal incision. A glass electrode of the condenser type having a capacity of 0.2 to 0.5 cc. was attached to the left ureter. For arterial pH a bulb type of electrode fitted into a cannula (Fruhling and Winterstein, 1933) was mounted in the left carotid artery of heparinized animals. Continuous records of both urine and blood pH were made on smoked paper as previously described (Brassfield, 1936).

To insure a flow of urine sufficient for these studies a constant intravenous injection of isotonic saline was maintained by a small injection pump. A Gibbs drop recorder delivering 25 drops per cubic centimeter was used to record the flow of urine from the right ureter. Mean blood pressure and pulmonary ventilation were recorded in the usual manner.

The urine after having passed through the glass electrode was collected under toluol in 1 cc. samples and immediately placed in a small icebox until the analyses could be made. The samples were analyzed for ammonia nitrogen by Conway's micro-ammonia method (1933), inorganic phosphate by Kuttner and Lichtenstein's modification (1930) of the Bell-Doisey method, and chloride by Sendroy's micro-chloride method (1937).

RESULTS. Preliminary experiments were performed to ascertain the

reliability of the continuous pH method.   In one series of experiments a glass electrode was attached to the cannulated ureter for recording continuously the pH values.   The urine flowing through the electrode was collected for pH determinations on a set adapted for single samples at 37°C. and the results plotted on the same record with the continuous pH curve.   A similar series was obtained with arterial blood.   Blood samples from the left femoral artery served to check the continuous blood pH curves.   Figures 2 and 1 are illustrative of these experiments.   The close
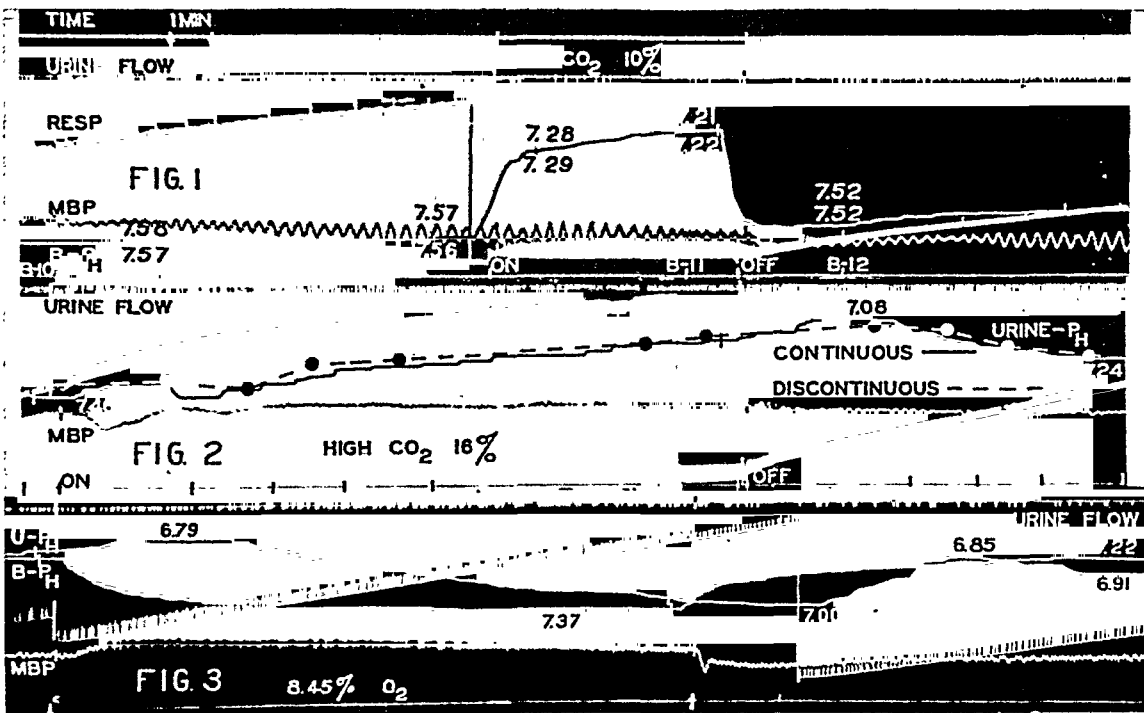
Fig. 1. Effect of 10 per cent CO_2 on arterial blood pH.   Numbers above the continuous blood pH curve (B-P_H) were obtained from the calibration of the continuous electrode; the numbers below the curve were obtained from samples taken and the pH determined on another electrode.

Fig. 2. Comparison of continuous and discontinuous urinary pH curves during and after the administration of 16 per cent CO_2.

Fig. 3. Record showing continuous pH curves for both urine and blood in a low oxygen experiment.

agreement between the continuous and discontinuous records indicates that the continuous method used here is a reliable indicator of the pH values.

Carbon dioxide mixtures.   Intra-tracheal administrations of carbon dioxide, oxygen, and nitrogen mixtures in which the carbon dioxide varied from 7 to 16 per cent, were made.   Results from 21 out of 26 procedures showed that a sudden decrease in blood pH was followed by a gradual decrease in urinary pH, which culminated at the end of the procedure

in a marked decrease of short duration followed by an immediate increase on return to room air. This marked decrease, occurring at the close of the procedure, was usually more prominent in cases where the urine flow was either very sluggish or stopped during the carbon dioxide administration, but increased in rate at the end of the procedure. Thus, the acid products accumulated during the carbon dioxide excess were not removed until urine flow was initiated on return to room air. In five instances, a slightly increased pH occurred in the urine just after the onset of the procedure, but previous to the gradual decrease in pH. Figure 2 is illustrative of these results. This record shows an administration of 16 per cent carbon dioxide for 17 minutes. The urine pH changed from 7.46 before the carbon dioxide administration to 7.08 two minutes after the close of the administration; then increased to a pH of 7.24 six minutes later. The blood change, which is not depicted here, but determined from arterial samples, was from a pH of 7.41 previous to the administration to a pH of 7.08 during the procedure. On return to room air, the blood pH increased to 7.38 within 60 seconds. Figure 1 is demonstrative of a continuous blood pH curve obtained by subjecting the dog to a 10 per cent carbon dioxide mixture. The sudden acid change, brought about in the blood, is shown by the sharp rise in the blood pH curve, which falls on return to room air to a pH 0.05 or 0.06 less than the pre-administration pH. In some cases a slight over-shooting has been observed, that is, the blood has attained a greater pH immediately following the administration, with a return to the pre-administration pH in a few minutes. Gesell and Hertzman (1926) noted this when following blood changes with the manganese dioxide electrode. The urine samples collected during the carbon dioxide administration recorded in figure 1 showed a pH decrease of 0.38 pH.

The blood pH changes observed varied between 0.12 and 0.38 pH, depending on the amount of carbon dioxide in the gas mixture. The urine pH changes were of a much greater magnitude, ranging from a pH change of 0.20 to one of 1.02.

Administration of carbon dioxide definitely slowed urinary secretion. In the 16 per cent CO_2 procedure (fig. 1) the rate was slowed from 28 drops per minute to 16.1 drops per minute. Return to room air increased the flow to 25.8 drops per minute. In some cases where the pre-administration urinary secretion was small the carbon dioxide produced anuria, as shown in figure 1, although the CO_2 concentration was only 10 per cent. These two procedures are illustrative of the results obtained on the rate of urine flow under carbon dioxide.

The latent period for the effects of CO_2 administration to alter the blood pH was as short as 5 seconds, whereas the urine pH curve required at least 50 seconds after the blood change appeared. In cases characterized by a very slow urinary output or a complete anuria, the change in the

urine curve did not appear until the flow was initiated or until the rate increased.   Thus due to the capacity of the space between the electrode and epithelium of the kidney the latent period of the urinary·pH change as recorded is much too long.

The micro-analyses made on the urine showed a chloride decrease and an ammonia and phosphate increase coincident with the decrease in urinary pH.   Data plotted in figure 5 are typical of the results obtained during 3
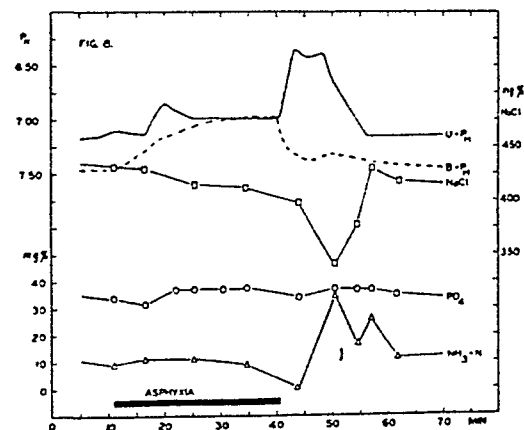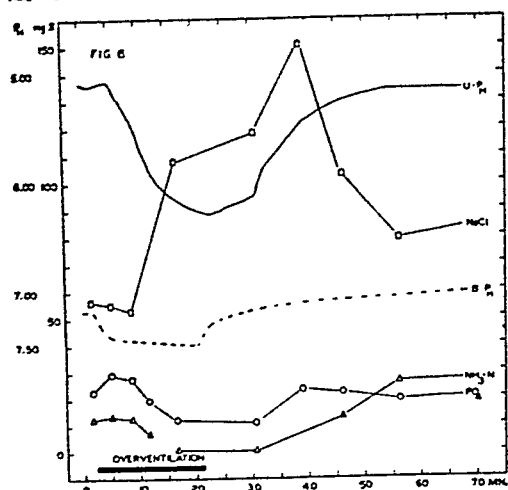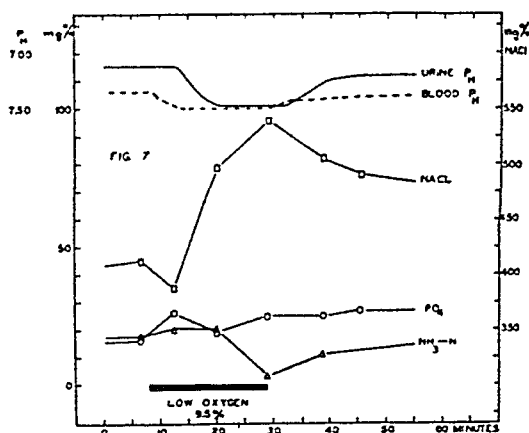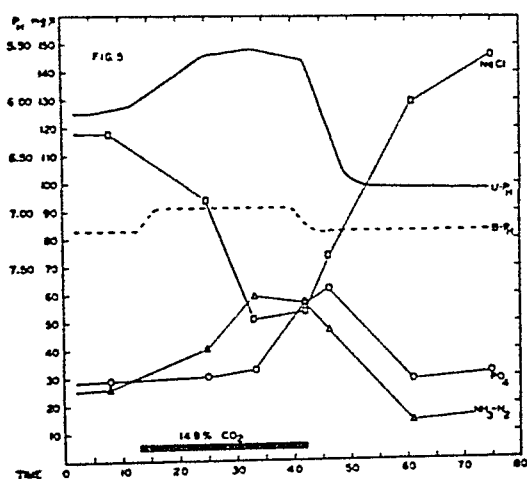
Figs. 5, 6, 7 and 8. Curves for urinary and arterial blood pH and urinary NaCl, PO₄ and NH₃-N during changes of pulmonary gases and ventilation.

carbon dioxide administrations.   It is to be noted that the least chloride excretion, and the greatest ammonia and phosphate excretion coincide with the lowest urinary pH.   These findings are in keeping with numerous reports on acid, ammonia, chloride and phosphate excretion with the exception of the data of Havard and Reay (1926), who noted no ammonia formation on administration of 6 to 8 per cent CO_2 to humans, nor did they observe any significant change in chloride excretion.

In reported experiments performed on man, diuresis usually resulted as a consequence of the administration of carbon dioxide. The anuria or decrease in urine formation which occurred in these experiments is in agreement with the results of Adolph (1935) on frogs. The actual mechanism of the depressed function in the anesthetized animal is as yet undetermined. The anesthesia may be a factor in bringing about the anuria. Considering the hypothesis of Adolph that local pressure changes may occur in the kidney as a result of carbon dioxide intoxication, being either a constriction of the afferent arterioles or a relaxation of the efferent arterioles or both, one may postulate that a similar circulatory change occurs in the region of the dog kidney.

Mechanical asphyxia. Mechanical asphyxia was produced by clamping with a large hemostat the rubber tubing connecting the trachea to the rebreathing tank. This was done at the end of an inspiration so that the lungs were filled with room air during the asphyxial period. In three cases, a progressive asphyxia was obtained by allowing the animal to breathe through the rebreathing system in which the volume of air was cut down to a few liters and the CO_2 allowed to accumulate.

Twenty-eight asphyxial experiments were performed on anesthetized dogs. In 8 of these there was no urinary secretion following the procedures. The length of time that the animals were asphyxiated varied from 40 to 90 seconds. The blood showed a decrease in pH within 10 to 20 seconds after the trachea was clamped and continued to decrease throughout the asphyxial period. At the end of each asphyxial period, a marked hyperpnea brought about an increased pH which gradually returned to the pre-asphyxial value. Immediate anuria was typical of all cases. As soon as the secretion started, the urine pH curve rose rapidly and then dropped toward the previous level. While the arterial blood always showed an acid increase within 10 seconds after the trachea was clamped, the urine pH change did not occur until the urinary flow was initiated. The marked change in the gradient of the curve as soon as urinary flow began, may be indicative of a diffusion of CO_2 into the urine formed previous to the administration. On the other hand, there may have been a greater reabsorption of basic substances in the tubules, to alleviate the state of acid excess. Progressive asphyxia resulted in a gradual decrease in both blood and urine pH.

Urinalysis for chlorides, ammonia and phosphate of the samples obtained immediately following the asphyxial period gave evidence of a decreased chloride excretion, an increased ammonia formation, but no appreciable change in phosphate elimination. Five out of 6 asphyxia experiments caused a decrease in chloride excretion, while one showed no apparent change. Ammonia formation increased in amounts ranging from 10 to 38 mgm. per cent. Phosphates did not change appreciably in

5 instances, although one case brought forth data showing an increase of 40 mgm. per cent after the asphyxial period was over.

In figure 8 are shown curves typical of progressive asphyxia. While the blood pH shows a gradual decrease throughout the entire period and reaches its lowest value at the end of the procedure, the urine pH, chloride and ammonia show the greatest change 8 minutes following the greatest blood change. As in CO_2 administration the lowest chloride value and the highest ammonia value coincide with the lowest urinary pH, which may be indicative of the hydrogen ion acting as a common stimulus in the two cases. The anuria could be brought about as a result of carbon dioxide and low oxygen acting separately or together upon the mechanisms controlling the flow of blood through the kidney.

Low oxygen. Intra-tracheal administrations of low oxygen mixtures (6–10 per cent) were made for periods of time ranging from 10 to 35 minutes. In 21 procedures the low oxygen produced an alkaline change in the blood, which shifted back to normal immediately on return to room air. The urine increased in pH also, but it was a gradual change. Although the magnitude of the urine pH change was usually more than twice that of the blood pH change, the gradual slope of the urine curve did not reach its limit until shortly after the procedure was ended. In 4 cases the urine showed an acid change previous to the alkaline trend, whereas 3 instances produced no significant alteration in the urine pH.

A $16\frac{1}{2}$ minute administration of 8.45 per cent oxygen (fig. 3) caused the typical results of a rise in blood pressure, a hyperpnea and a diminution in the urinary flow. The rate decreased from 14 drops/minute to 10 drops/minute during the administration, with a rate of only 6 drops/minute on return to room air. Results were obtained in which the recovery, as regards rate of flow, was both incomplete and complete. In this administration of low oxygen the blood increased 0.15 pH and showed an exact reversal on return to room air. The urine pH increased from 6.79 four minutes after the onset of the administration to 7.00 three minutes after the return to room air, whereupon it decreased to 6.85 within the next four minutes.

It was noted that the lower the oxygen percentage, the greater were the changes in the blood and urine pH. The effect of the anoxia on the blood pH was usually seen within 10 seconds after the onset of the procedure. The urine, however, usually required 2 to 5 minutes before the alkaline trend occurred. The rate of flow was undoubtedly a factor in preventing the urine pH change from appearing on the record as soon as it occurred in the kidney.

Urinalysis was made on 6 low oxygen procedures. From the data obtained it appeared that the phosphates showed no significant change in 5 procedures. One period of anoxia decreased the phosphate elimination.

Ammonia formation fell in 5 out of 6 administrations while one low oxygen procedure caused no change in the rate of ammonia formation. The chloride excretion rose in 3 out of 3 cases. A graphic representation of results in a 9.5 per cent low oxygen administration for 20 minutes and 50 seconds as shown in figure 7 demonstrates a blood pH increase of 0.14 and a urine pH increase of 0.33 pH. Both curves reversed on return to room air. Urine formation in this procedure decreased from 11.5 drops/minute to 7.0 drops/minute during the anoxia. Recovery increased the rate to 12 drops/minute. The urinary constituents showed the typical directional changes of low oxygen. The lowest ammonia and the highest chloride values were determined from the sample which corresponded to the most alkaline pH. These findings on urinary constituents are similar to those found in humans by many investigators.

It appears that the results, which Toth (1937) obtained in his study of anoxia in dogs, showed a parallelism between chloride and water excretion. In the anesthetized sacrifice dogs, an oliguria developed, with an accompanying low chloride excretion. Unanesthetized bladder-fistula dogs responded to low oxygen with a polyuria and a coincident rise in urinary chloride. Toth suggests that anoxemia may result in an asphyxia of the tubular epithelium, with a consequent interference with the process of reabsorption. This would entail a polyuria approaching plasma composition. His experiments, however, did not show a urine of plasma composition on initiation of polyuria. The low urinary rates in our experiments, accompanying the high chloride excretion, indicate a lack of correlation between water and chloride excretion in anoxemia on the anesthetized dog.

Overventilation. Overventilation was produced by an electrically driven pump in closed circuit with the rebreathing tanks. Fifteen procedures of varying duration (5–30 min.) were performed. The blood always showed an alkaline trend varying from 0.18 to 0.32 pH depending upon the magnitude of the ventilation. The majority of the procedures caused an immediate increase in the urine pH, accompanied by a diuresis. In 4 cases, the urine offered varied changes during the procedure, but has consistently shown a marked alkaline change after the overventilation. A decreased urinary flow may have been a factor in the recording of these results.

A graphic representation of a hyperventilation procedure (fig. 6) which induced an increase in urine formation, demonstrates the typical results. The rate of flow in this 20 minute procedure increased from 11 drops/minute to 15 drops/minute. At the close of the procedure the rate fell to 5 drops/minute. The blood pH began to increase from 7.23 to 7.50 within 10 seconds after the hyperventilation was begun while the urine had a latent period of 1 minute and 50 seconds before the pH began to increase

from 5.08 to 6.28.   Recovery shows that both curves returned to approximately the same pre-administration level.

Characteristic changes in the urinary constituents are plotted on the chart.   In each of 6 procedures, the chlorides showed an increase during the overventilation, although the degree of the rise varied with the duration and the degree of the hyperpnea.   Ammonia formation and phosphate elimination decreased in 4 cases, while 2 procedures elicited no appreciable change.   In the 2 cases in which the ammonia and phosphate were unaffected by the overventilation, the urinary pH changes were less prominent.   Usually the most alkaline part of the urinary curve occurred simultaneously with the depression in ammonia and phosphate excretion and the increase in chloride elimination.

If one compares the charts, typical of low oxygen (fig. 7) and overventilation (fig. 6) it is easily seen that overventilation creates in all respects except urinary flow a similar picture to low oxygen effects.   The total hyperventilation (56.7 liters/kgm./hr.) caused a blood pH change of 0.27 pH while the total ventilation induced by low oxygen (33.9 liters/kgm./hr.) changed the blood 0.14 pH.   The urine of the hyperventilated animal increased 1.20 pH while that of the dog subjected to anoxia increased 0.33 pH.   Phosphate elimination, though unaffected in the low oxygen procedure was found to decrease 19 mgm. per cent under overventilation.   Ammonia formation fell to a level of 3 mgm. per cent under low oxygen and to zero under overventilation.   The chloride rose 152 mgm. per cent under low oxygen and 103 mgm. per cent under the hyperventilation.   Rate of urinary flow always decreased in the anoxial state but in the majority of the overventilation experiments it increased.   The decreased rate of urine formation, characteristic of low oxygen is undoubtedly due to the low oxygen effects on the kidney.   In the light of the work of Adolph (1936) and Toth (1940), these are purely of a circulatory nature. In overventilation, an abundance of oxygen prevents such an oliguria.   In fact, a diuresis usually occurs.

SUMMARY AND CONCLUSIONS

Continuous blood and urine pH tracings under conditions produced by changes in pulmonary ventilation were recorded in the anesthetized dog.   Results show a qualitative similarity between blood and urine pH, the extent of the change being smaller and of shorter duration in the blood than in the urine.

A study of the latent periods shows that the blood pH reacts to pulmonary ventilation changes within 5 to 20 seconds while the urine pH, requires a variable length of time after the blood change occurs.   If the rate of flow through the electrode is rapid, the change may appear within

a minute; however, a slow flow or an oliguria lengthens the time before the urine electrode records the change.

Carbon dioxide and mechanical asphyxia caused a blood and urine pH decrease while low oxygen and overventilation produced an increase in blood and urine pH.

Ammonia formation, that mechanism of the kidney whereby base is conserved for the organism, was stimulated as a result of carbon dioxide and mechanical asphyxia. Low oxygen and overventilation produced an alkaline blood and suppressed the excretion of ammonia by the kidney.

Carbon dioxide administration caused an increase in the elimination of phosphates while that of mechanical asphyxia did not alter the urine phosphate materially. Overventilation caused a decrease in phosphate elimination while the alkaline state created by low oxygen was not sufficient to affect the amount of phosphate in the urine.

Chloride excretion was diminished under carbon dioxide and asphyxia, but increased markedly in the case of low oxygen and overventilation. Chloride diminution occurred in those cases where the phosphate elimination increased. This may be indicative of an inverse relationship between chloride and phosphate. Asphyxia, carbon dioxide and overventilation show chloride excretion changes, which are in the same direction as the rate of water excretion. In these cases, there may be a definite relationship between the chloride and the water excretion. Administrations of low oxygen show no correlation between water and chloride elimination.

Since the urine pH changes in the same direction as that of the blood and within such a short time after the blood changes together with the fact that the urinary constituents studied change in a direction such as to counteract the pH change of the blood seems to indicate that the hydrogen ion is the factor primarily concerned in these studies.

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FACTORS IN THE ABSORPTION OF INSULIN FROM THE ALIMENTARY TRACT[1]

R. L. DRIVER[2] AND J. R. MURLIN

From the Department of Vital Economics, University of Rochester, Rochester, New York

A number of reports from this laboratory (1, 2, 3) and others (4, 5) leave no doubt that insulin can be absorbed from the G. I. tract, but in no case has this absorption reached a practical amount (6), nor has a satisfactory explanation yet been given for the process of absorption of so large a molecule.

The object of this investigation was to find compounds and combinations of compounds that affect the absorption of insulin and to determine which, if any, of the well-known factors that in general influence absorption are involved in the case of insulin.

Effect of sodium amytal on the blood sugar of the dog. Since the sacrifice animals used were anesthetized with sodium amytal and the blood sugar was taken as an indication of the extent of the insulin absorbed, the effect of sodium amytal alone on blood sugar was studied. All of the animals used in these control experiments were subjected to operation and treated exactly as were the experimental animals with the exception that an isotonic solution of sodium chloride was placed in the isolated loop instead of a solution containing insulin. Blood glucose determinations were made by the method of Shaffer and Hartman as described by Shaffer and Somogyi (7), using the zinc precipitation procedure of Somogyi (8). Two dogs were given the amytal intravenously and intramuscularly in the ratio of 2:1. In three dogs the amytal was given intraperitoneally. The blood sugar changes of these five dogs are shown in figure 1.

In general there is a gradual falling off in blood sugar with time, amounting to an average of about 15 mgm. per cent in 12 hours. Therefore, to minimize corrections, the data on insulin absorption are divided into two groups: 1, the blood sugar changes when insulin was given within 5 hours after anesthesia, and 2, the changes after 5 hours following anesthesia.

Response of the amytalized dog to known quantities of insulin. In order

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[1] Taken from a thesis submitted by R. L. D. in partial fulfillment of the requirements for the doctorate of philosophy, University of Rochester, June, 1940.

[2] Present address: Dept. of Anatomy and Physiology, University of Kentucky, Lexington, Ky.

to get some indication of the amount of insulin absorbed from the G-I tract, known quantities were given to the amytalized dogs subcutaneously and intravenously. The results given in figure 2 show that consideration must be made of the level of blood sugar at the time of administration; and in the experiments following, all data accumulated when the initial blood sugar was below 50 mgm. per cent were discarded.

Absorption of insulin in sacrifice dogs. (a) *Compounds and combination of compounds that have an effect.* The technique previously used (17, 3) of introducing insulin with test substances into a segment embracing the lower duodenum and upper jejunum of the anesthetized dog has been continued. The solutions used in this group of experiments uniformly had a pH of 4.5. The effect on blood sugar of the various solutions is
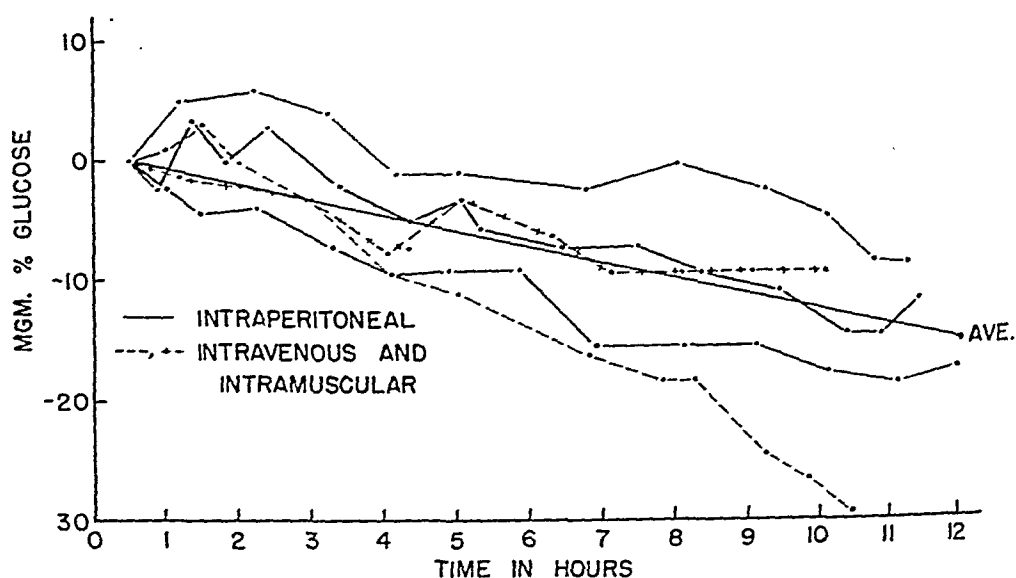
Fig. 1. Changes in blood sugar of dogs under amytal anesthesia

shown graphically in figure 3, where the upper bar with each entry indicates the average blood sugar fall induced by the solution when given within 5 hours after anesthesia, while the lower bar represents the fall 5 or more hours after anesthesia. One-half hour was allowed for absorption.

An examination of the data reveals that absorption falls off with time after anesthesia, and this factor must be considered in studying the data.

Comparing single compounds, we find the following order of *effectiveness*: pinacol (tetramethyl glycol) and methyl salicylate, thiamin, quinine and hexyl resorcinol, Aerosol O.T. (dioctyl sodium sulfosuccinate), and calgon (sodium hexametaphosphate). The reports of other workers on the effectiveness of pinacol (4), hexyl resorcinol (2) and quinine (Cutting, private communication) are confirmed, and methyl salicylate, thiamin, Aerosol O.T. and calgon are added to the list.
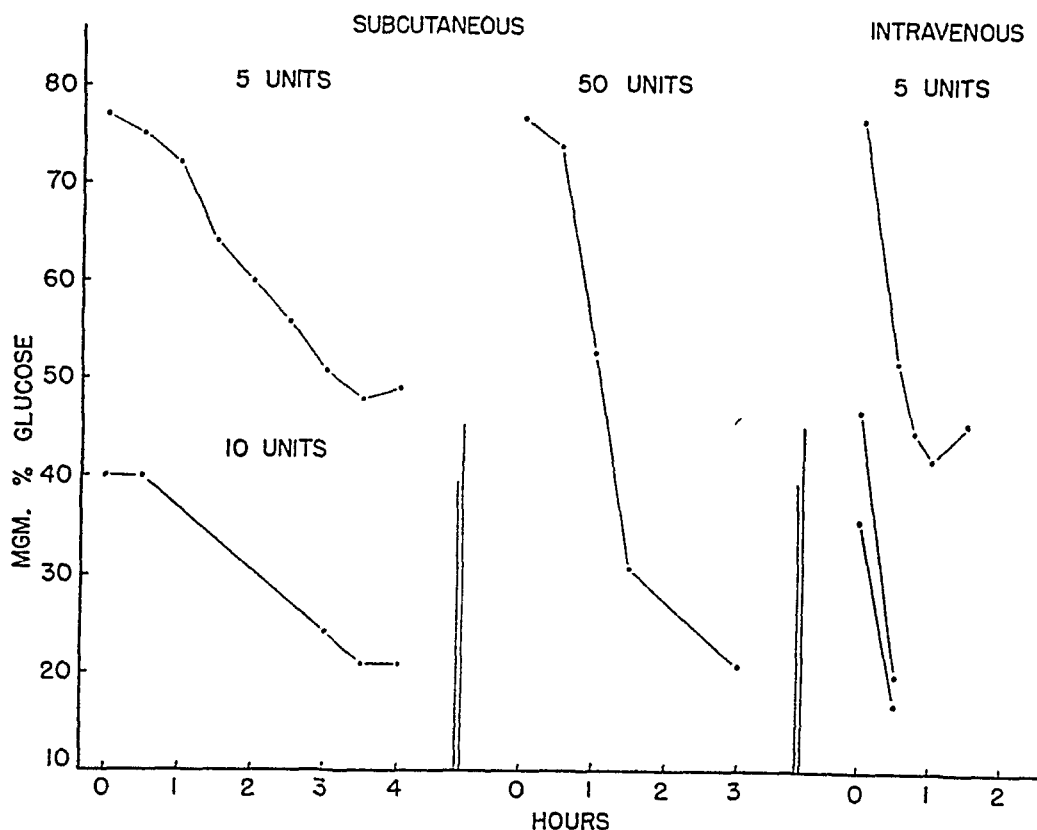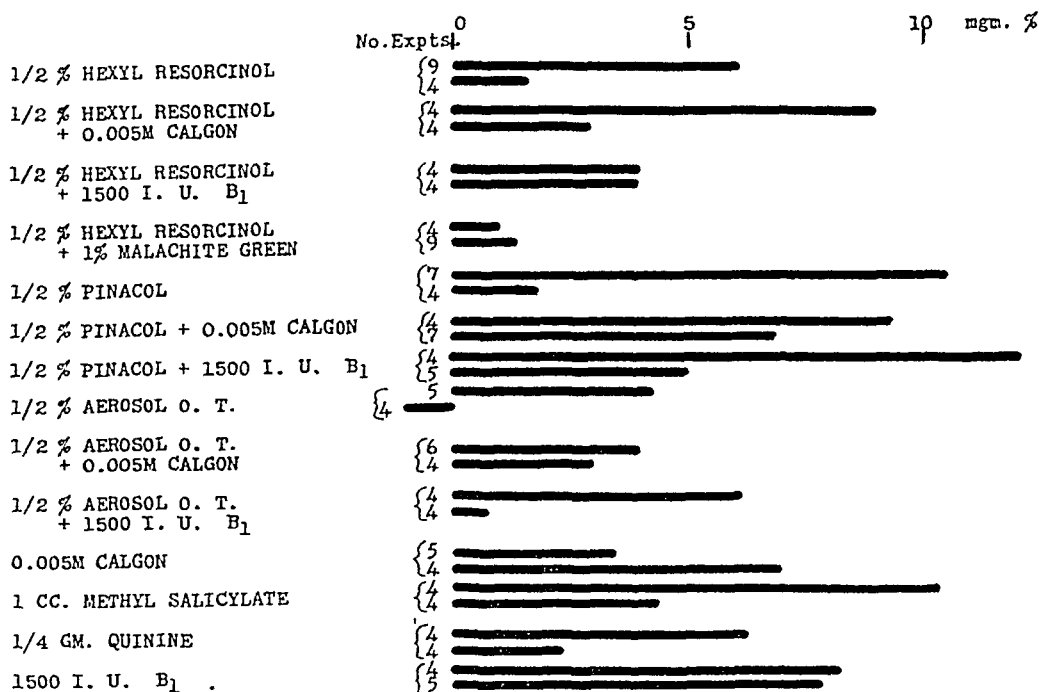
Fig. 2. Response of the amytalized dog to insulin

Fig. 3. Fall in blood sugar from 50 I. U. insulin in intestinal loop

The influence of methyl salicylate is in line with the observation of Lauter (private communication) that this reagent promotes the absorption of the alkaloids, morphine and strychnine, by the skin. The compound is quite irritating and its action may be due to an increased circulation in the intestinal mucosa.

The effect of thiamin may not have been due entirely to absorption, for Mosonyi and Aszódi (9) demonstrated that the intravenous injection of vitamin B_1 into normal men lowered the blood sugar 20 to 40 mgm. per cent and increased the insulin in the blood. However, in the experiments reported here, thiamin must have had some effect on absorption because when it was given alone the results were negative.

Calgon is a compound very powerful in its ability to tie up calcium ions (10). Since this ion has been reported to inhibit absorption (11, 12, 13, 14, 15), it was postulated that its removal would be conducive to an increased absorption of insulin. The assumption was borne out to a small extent only.

The good response to pinacol, which does not lower surface tension, and the poor response to Aerosol O.T., which does, confirm the observation (3) that surface tension, per se, cannot account for an increase in absorption.

A combination of compounds influencing absorption does not always show an additive effect. It is questionable whether one could always expect a summation, because it is not inconceivable that some of these compounds may react with each other to the detriment of both, even though no precipitates were seen at any time.

(b) *Substances without effect on the absorption of insulin.* Some substances that could conceivably promote the absorption of insulin from the gastro-intestinal tract and the reasons for trying them are listed in table 1. In the first group there are 28 different compounds having hydrophobic and hydrophilic groups, chosen with the view in mind of finding one with a structure such that it would fit into the insulin molecule by a sort of lock-and-key mechanism, in the manner postulated in the case of the bile salts and fatty acids (16). A logical procedure would be to hold, say, the hydrophilic group constant and vary the hydrophobic group until the best effect should be found, and then reverse the experiment until the most effective hydrophilic group should be discovered. But it was found that the two groups are interdependent, and one might as well follow a hit or miss procedure.

The importance of electrolyte ions in the absorption of other substances would seem to be of little consequence in the case of insulin. The addition of potassium to the solution gave negative results and the tying up of calcium with sodium citrate was likewise ineffective.

Since methyl salicylate, a mucous membrane-disturbing compound,

definitely promoted the absorption of insulin, other substances which irritate the intestine should behave similarly. However, this was not found to be true, and it appears that a mere change in the mucosa is insufficient: it must be one of specific character. Not even an increase in villi action will influence insulin absorption.

TABLE 1

Substances without effect on absorption of insulin grouped under reasons for trying them

Hydrotropic or lower surface tension	*Tie up calcium ion*
Tetramethyl ammonium hydroxide	Sodium citrate
dl-malic acid	
Diethyl acetic acid	*Have an irritating effect on or change*
Malonic acid	*character of the intestine*
Butyric acid	Saponin
Rochelle salt	Histamine phosphate
Lactic acid	Aspirin
Succinic acid	Strophantin
Calcium lactate	Yeast
Sodium cetyl sulfate	Ernutin
Aerosol M.A.	Digitonin
Aerosol O.S. sodium alkyl naphthalene	
sulfonic acid	*Potassium aids absorption*
Sodium glycocholate	Potassium chloride
Keto ethylresorcinol	
Keto propylresorcinol	*Increase villi action*
Keto butylresorcinol	Oil of clove
Keto pentyl resorcinol	Zinc sulfate
Butyl-b-resorcylate	Yeast
Butylsalicylate	
Butyl-p-hydroxybenzoate	
2,4-dihydroxybenzoic acid	
n-butyropyrogallol	
p-ter-butyl-phenol	
Tyrosine	
Salicylic acid	
Orcinol	
Thymol	
Amyl cresols	

Relation between surface tension and absorption. A large number of investigators have attached importance to surface tension as a factor in absorption, and the literature on the subject is too vast to attempt a review here. An examination of table 2 with compounds arranged in the order of decreasing effect on surface tension seems to dispose finally of the idea that this property is in some way closely related to the absorption of insulin (17).

Absorption of insulin in normal dogs. In these experiments the solution

containing insulin and the compounds to be tested were introduced into dogs' stomachs by a stomach tube. Samples of blood were taken before giving the solution and also one a half-hour and one an hour after administration. Eighty-seven experiments were done on eight normal dogs, about

TABLE 2

Effect of surface tension on absorption of insulin

SOLUTION	BLOOD SUGAR DROP	SURFACE TENSION
	mgm. %	*dynes/cm.*
½ per cent hexyl resorcinol......................	6	27
½ per cent Aerosol O.T...........................	4.3	29
½ per cent Aerosol M.A...........................	0	37
½ per cent Aerosol O.S...........................	0	39
Saturated sodium cetyl sulfate....................	0	45
½ per cent saponin...............................	0	63
½ per cent sodium benzoate.......................	0	66
½ per cent pinacol...............................	10.4	66
0.005 molar butyl salicylate......................	0	68
0.005 molar calgon...............................	3.4	68
Water...	0	70
1500 I. U. B_1 (water solution)....................	8.2	72
0.005 molar butyl-b-resorcylate...................	0	73

TABLE 3

Compounds having no effect on the absorption of insulin when given by stomach tube

COMPOUND	NUMBER OF EXPERIMENTS
Trypan red......................................	2
Urea..	2
Saponin...	4
*Vitamin B_1....................................	4
*Aerosol O.T....................................	2
Benzoic acid....................................	2
Lactic acid.....................................	2
Starch..	2
Ferric hydroxide................................	2
Gum arabic......................................	3
*Quinine..	4
Sodium citrate..................................	8
Malachite green.................................	4
Oil of clove....................................	2

* These compounds are effective in an isolated loop.

fifty of which were done on two dogs. The concentrations of the various substances used ranged from 0.1 to 1 per cent, the amount having little effect on the results. The substances tried under conditions which gave good responses with other substances, and therefore believed to have no effect in this type of experiment, are given in table 3.

In many cases the findings in the loop experiments were confirmed, while in others they were not. The mere presence of a substance which lowers surface tension has no effect, nor do the removal of calcium, increased villi action, or most intestinal irritants bring about an increased absorption. Malachite green and other substances supposed to inhibit enzyme activity (5) have no effect superimposed upon the effect of those

TABLE 4

Positive stomach tube experiments

	NO. OF EXPTS.	BLOOD SUGAR DROP	
		½ hr.	1 hr.
		mgm. per cent	*mgm. per cent*
Effect of pH:			
*pin + cal (pH 3.5).....................	1	+8	+6
pin + cal + buf (pH 10.1)..............	2	8	8
pin + hex + cal (pH 4)................	6	6	2
pin + hex + cal + buf (pH 10)........	9	12	4
Effect of serum:			
pin + hex + cal + buf................	9	12	4
pin + hex + cal + buf + serum.......	2	29	24
Effect of hex:			
pin + cal + buf.......................	2	8	8
pin + hex + cal + buf................	9	12	4
Effect of pg:			
pin + hex + cal + buf................	9	12	4
pin + hex + cal + buf + pg...........	2	31	15
Effect of ms:			
pin + hex + cal + buf................	9	12	4
pin + hex + cal + buf + ms...........	3	22	20
Effect of composite:			
pin + hex + cal + buf + ms + pg......	6	26	12

* hex = hexyl resorcinol; pin = pinacol; cal = calgon; buf = a sodium carbonate-bicarbonate buffer that will give the solution introduced a pH of about 10; pg = propylene glycol; ms = methyl salicylate.

compounds which give positive experiments. On the other hand, thiamin, Aerosol O.T. and quinine were found to be without action in the stomach tube experiments, but were definitely positive in the isolated loop. It must be remembered that these compounds were given in a solution containing other substances and a combination may have taken place rendering them useless.

The data in table 4 are presented for the experiments in which there are

no complicating outside influences other than the presence at times of one of the above-mentioned noneffective compounds.

It was thought best here to make preliminary experiments on a number of substances and try to build up an effective combination rather than spend too much time on a single questionable factor. Table 4 was developed following this procedure, which is justified by the progressive improvement shown. Clearly it is possible to build up a combination which summates the effects of different compounds. This summation was not always found, of course, as was also the case in the loop experiments.

Enzyme studies. These investigations were prompted by the observations of Daggs, Murlin and Murlin (18) that hexyl resorcinol protects insulin from hydrolysis by pepsin and of Young, Phillips and Murlin (17) that the compound inhibits the activity of trypsin. The effect of

TABLE 5

Enzyme studies

	PEPSIN ALBU-MIN DIGESTED	TRYPSIN N/10 ALKALI	EREPSIN N/10 ALKALI
	mm.	*cc.*	*cc.*
Control..........................	4.5	6.9 7.2 > 7.1	3.7 3.1 > 3.4
½ gram quinine.....................	2.0	6.6 5.8 > 6.2	3.7 3.0 > 3.4
1 cc. 5 per cent pinacol.................	4.5	6.0 6.6 > 6.3	3.8 3.6 > 3.7
1 cc. 10 per cent calgon................	0.0	6.9 7.2 > 7.1	3.2 3.3 > 3.3
0.1 gram hexyl resorcinol..............	0.0	4.6 4.3 > 4.5	1.7 1.8 > 1.8

some of the substances that promote the absorption of insulin on pepsin, trypsin and erepsin activity was investigated, and the results are compared in table 5. The procedures used were as follows:

Pepsin studies. Mett tubes were incubated at 37°C. in 30 cc. solution of 2½ per cent Bausch and Lomb flake pepsin in N/20 HCl for 30 hours. The amount of albumin digested was measured. The figures in table 5 are the average result of 4 determinations.

Trypsin studies. Twenty cubic centimeters of 1¼ per cent fat-free casein plus 10 cc. of Baker and Adamson trypsin plus 0.5 cc. of toluene were incubated for 32 hours. A 10 cc. aliquot was taken to which was added 10 cc. of neutral formaldehyde and 100 cc. of water. The solution was titrated with N/10 alkali.

Erepsin studies. Ten cubic centimeters of 2½ per cent bacteriological peptone plus 20 cc. of 5 per cent Difco erepsin plus 0.5 cc. toluene were treated exactly as described above.

Quinine, a protoplasmic poison, was shown by Cutting (private communication) to promote the absorption of insulin in the intestine of rabbits,

and this observation was confirmed in the isolated loops of dogs. Although the compound inhibits pepsin activity, it proved ineffective in stomach-tube experiments, where this activity would be important, and was effective in intestinal loop experiments where it could not be important. Presumably its action in the dog is exactly the same as in the rabbit.

Pinacol has no appreciable effect on any of the enzymes while calgon inhibits the activity of pepsin. These facts do not furnish any explanation of the absorptive activity previously noted, of the substances named.

The most important observation is that hexyl resorcinol inhibits the activity of all three enzymes and furnishes insulin protection from destruction, and this no doubt explains in part at least its efficacy in promoting the absorption of insulin.

DISCUSSION. The results of control experiments indicate that sodium amytal is a suitable anesthetic for the type of work reported here, and indicate how readily correction may be made, in consideration of the effect of the anesthetic, in interpreting the results. The blood sugar of the dog shows a gradual falling off with time after the administration of the anesthetic, and this change can be easily taken into account. The response of the animal to known amounts of insulin shows that, providing the blood sugar is above 50 mgm. per cent, 5 units injected intravenously will bring about a drop of about 20 mgm. per cent in 30 minutes. Using 50 units subcutaneously in an animal of comparable size, an effect but little greater is obtained in this interval of time. Obviously it would be unfair to expect as great a change from alimentary administration as from intravenous. But many experiments show quite as great an effect from the intestinal loop washed free of enzymes and treated with absorptive agents as from subcutaneous injection of the same dose, within the same time.

It is conclusive now that the absorption of insulin is not related to surface tension and hydrotropism, but involves problems which have been inadequately investigated. For example, the calcium ion has been reported to inhibit absorption, but the studies from which this inference was drawn were limited to simple substances like salts and sugars. In this work calgon had a slight effect, but citrate did not, and it is likely that removal of the calcium ion has no effect on the absorption of insulin.

The irritation of a membrane or an increased blood flow will not in themselves bring about an increased absorption of insulin. The effect on the mucosa must be specific because quinine, a "protoplasmic poison," and methyl salicylate were active in isolated loops whereas other irritants were not.

Perhaps of more interest have been consistent reports from this laboratory that a high pH favors absorption. Young, Phillips and Murlin (17) found that the optimum pH range for maximum effect in stomach tube experiments was from 9.9 to 10.5. The data in table 4 show that a high pH is favorable for absorption, and since these data were obtained from

stomach tube experiments, it may be argued that the increased absorption of insulin was due to a protection of the insulin from pepsin by alkali. But Sealock, Murlin and Driver (3) using hexyl resorcinol at a pH of 10 in intestinal loops found an average blood glucose change of 10.3 mgm. per cent compared to a drop of 6 reported in this paper where the pH of the solution was 4.5. Since no pepsin was present in these loop experiments, it seems that the alkali brings about some kind of a change in the mucosa, perhaps nothing more than the familiar softening effect on tissues generally.

Since the efficacy of hexyl resorcinol cannot be explained on a surface tension basis, it is necessary to look for other factors. A comparison (3) of the alkyl resorcinols in their effects on absorption and bactericidal action showed a perfect parallelism between the two, hexyl resorcinol being most effective in both cases. What can be inferred from this parallelism? Höber, Andersh, Höber and Nebel (19) found that hexyl resorcinol de-polarized membranes of muscle and nerve. Osterhout (20) found the same thing with Nitella and a simultaneous decrease in the apparent mobility of sodium and potassium. He stated that hexyl resorcinol probably produces structural changes in Nitella, but that the nature of these alterations required further investigation. Höber et al. described the effect of hexyl resorcinol on membrane potential as due to a dispersing effect on the colloids of the surface. The investigations reported here also indicate a change in the membrane. Preliminary results indicate that a combination of hexyl resorcinol with alkali completely reverses a hindering effect of hexyl resorcinol alone on dead or surviving membranes. This combination is always more effective in promoting absorption than the resorcinol in acid medium. Hence, it may be inferred that a still greater "dispersing effect" is caused by the combination, or dispersal plus softening (see above). Probably the combination would have also a greater bactericidal effect.

Pinacol has no appreciable effect either on surface tension or enzyme inhibition, and yet it is one of the most effective agents known in promoting the absorption of insulin. This property is probably due to a favorable combination of hydrophobic and hydrophilic groups in the molecule which enable it to fit into the insulin molecule by a sort of lock-and-key mechanism. It was thought that the hydrophobic groups attached themselves to the insulin molecule leaving the two polar hydroxyl groups exposed exteriorly, thereby making the insulin more soluble and allowing it to penetrate the aqueous part of the membrane more rapidly as has been postulated for the action of bile salts on fat absorption. In order to test this premise, the influence of pinacol on the diffusion of insulin through a sintered glass membrane was studied, but there was no increase in diffusion as there should have been if the hypothesis were correct. Now since at least 30 hydrotropic substances have been found which are without effect,

the question arises as to whether the absorption of insulin is a matter of water solubility alone. Future experiments will explore the possibility of effects from agents which modify lipoid solubility.

SUMMARY

The blood sugar of the dog under amytal anesthesia falls gradually, amounting on the average to about 15 mgm. per cent in 12 hours.

In many experiments with intestinal loops effects on blood sugar quite as great as from subcutaneous administration have been obtained in 30 minutes from the same amount of insulin introduced into the loop with various promoting agents. However, the effect is shorter-lasting, which means obviously that less insulin reaches the circulation from the alimentary tract. Pinacol, Aerosol O.T., methyl salicylate, thiamin and quinine, as well as the alkyl resorcinols, promote the absorption of insulin in this manner at a pH of 4.5. The effect of methyl salicylate is attributed to its special irritating influence on the mucosa. The pinacol molecule probably has a configuration such that it fits into the insulin molecule, thereby bringing about a change in the penetrability of the latter. Alkaline reactions favor absorption still more. Quinine "poisons" the mucosa, whatever this may mean, causing a more rapid penetration of insulin.

The opinion has been reached that there is no direct relationship between the absorption of insulin and surface tension.

A high pH is advantageous in inhibiting the destructive action of pepsin on insulin and in softening the mucosa for its absorption.

Acidic and basic organic dyes and other colloids supposed to precipitate and inactivate enzymes do not promote the absorption of insulin when added to solutions containing hexyl resorcinol.

No effect on absorption is observed by increasing villous activity, adding potassium or removing calcium.

It is possible to obtain an additive effect on absorption by combining different factors.

Hexyl resorcinol promotes the absorption of insulin principally by changing the nature of the membrane and by protecting the hormone from enzymatic hydrolysis.

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A COMPARATIVE STUDY OF EXCISED CEREBRAL TISSUES OF ADULT AND INFANT RATS[1]

H. E. HIMWICH, P. SYKOWSKI AND J. F. FAZEKAS

From the Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, New York

Previous studies (1) from this laboratory have disclosed that the excised cerebral tissue of infant rats 1 to 25 days old possesses a lower oxygen consumption than adult tissue.   In the present investigation these results have been extended to include a comparative study of various parts of the adult rat brain, as well as of the effects of alcohol and pentobarbital on adult and infant cerebral tissue.

METHOD.   The oxygen consumption of minced cerebral tissues was measured in the Warburg respirometer.   The tissue was suspended in saline buffered with phosphate at pH 7.4 and glucose as substrate.   The brain of the adult rat was separated into four parts: cerebral cortex, cerebellum, medulla, and the remainder including basal ganglia, thalamus, hypothalamus, and midbrain was termed brain stem.   These parts were minced and white and gray matter were thus studied together.   The entire brain of the newborn rat (less than 24 hrs. post natal) was used. The various parts of the adult and the entire infant brain were exposed in different experiments to 6 per cent alcohol and 0.012 per cent pentobarbital.

RESULTS.   The data presented in tables 1 and 2 reveal that among the various parts of the adult rat brain, the cerebral cortex possesses the highest oxygen uptake (column 3).   Brain stem, cerebellum, and medulla follow in the order given.   The differences in the metabolic rate are significant.

The effect of 6 per cent alcohol (table 1) is to depress the metabolism of every part of the brain.   The cerebral cortex suffers the greatest absolute depression while that of the brain stem is the least (column 5 = column 3 − column 4).   On a percentage basis, cortex, cerebellum, and medulla are inhibited approximately to the same extent and brain stem to a lesser degree (column 6).

Pentobarbital in 0.012 per cent solution (table 2) exerts the most profound absolute depression on the cerebral cortex (column 5 = column 3 −

column 4). The percentage inhibition of the brain stem is less than that of the other parts of the brain (column 6).

TABLE 1

The effect of alcohol on cerebral metabolism of various parts of the adult rat brain

mm.^{3} O$_2$/100 mgm. tissue/hour

1	2	3	4	5	6
				DEPRESSION	
PART	NO. OF OB-SERVATIONS	CONTROL	ALCOHOL	Absolute	Per cent
Cortex......................	19	316	195	121	38
Brain stem..................	20	251	204	47	19
Cerebellum..................	20	179	111	68	38
Medulla.....................	20	161	103	58	36
Total....................	79				

TABLE 2

The effect of pentobarbital on various parts of adult rat brain

mm.^{3} O$_2$/100 mgm. tissue/hour

1	2	3	4	5	6
				DEPRESSION	
PART	NO. OF OB-SERVATIONS	CONTROL	PENTO-BARBITAL	Absolute	Per cent
Cortex......................	40	278	170	108	38
Brain stem..................	39	226	163	63	28
Cerebellum..................	40	167	106	61	37
Medulla.....................	41	147	90	57	39
Total....................	160				

TABLE 3

The effect of alcohol and pentobarbital on the infant rat brain

mm.^{3} O$_2$/100 mgm. tissue/hour

1	2	3	4	5	6
				DEPRESSION	
NO. OF OBSERVATIONS	CONTROL	ALCOHOL	PENTO-BARBITAL	Absolute	Per cent
16	102	81		21	21
44	105		83	22	22

The oxygen consumption of the brain of rats less than 24 hours old is approximately two-thirds that of the adult medulla (table 3, column 2). Both on a percentage basis as well as absolutely the depressive effects of alcohol and pentobarbital are less in the newly born rats (columns 5 and 6).

DISCUSSION. These results on the rates of metabolism of the various parts of the adult rat brain may be compared with those of Dixon and Meyer (2) on ox brain. The English workers observed the highest rate in the cerebellar cortex and then, in decreasing magnitude, the caudate nucleus, cerebral cortex, thalamus, hypothalamus, and Globus Pallidus. In Dixon and Meyer's experiments the gray matter only was studied and the difference observed in their experiments represented the rates of respiration of gray matter in the various parts of the brain. In the present experiment entire parts consisting of white and gray matter were studied. White matter possesses a much lower rate of metabolism than does the gray. Certainly the medulla with its low oxygen uptake possesses relatively the highest proportion of white matter. It is interesting that despite the difference in experimental materials, with the exception of the cerebellar cortex, the result of Dixon and Meyer in the main agrees with the present observation for the oxygen utilization of the cerebral cortex is greater than the average for the various parts of the brain stem.

An analysis of the oxygen consumption of the various parts of the brain suggests that in general there is an increase of metabolic rate as the neuraxis is ascended. The layers of newer phylogenetic origin appear to possess a greater metabolism. The lower metabolism of the immature brain of the newly born rat is in accordance with this conception. Similar observations have been made on newly born dogs (3). Many differences between infant and adult brain may be partly explained by this lower cerebral metabolism; for example, the greater resistance to cerebral anemia of the infant is probably associated with this smaller oxygen requirement (4). It has also been found that the cerebral electrical activity stimulated in the kitten by metrazol persists during a longer period of cerebral anemia than the activity of the adult cat (5). Perhaps the larger dose of metrazol required to stimulate convulsions in the kitten may also be associated with a lower cerebral metabolism.

Since the metabolic rate of each part of the adult rat brain appears to be characteristic, it was possible to make a comparative study of the effects of narcotics on the various cerebral fractions. There has been much discussion as to the site of action of narcotics, some of them have been classified as exerting their effects chiefly on the cerebral cortex and others on the brain stem (6). Alcohol is regarded by this worker as a cerebral narcotic and the present results reveal that the cerebral cortex suffers the greatest depression by alcohol. However, it should be pointed out that the concentration of alcohol used in these experiments is greater than the toxic dose. This indicates that *in vivo* alcohol produces its effects in other ways in addition to the depression of respiratory metabolism of cerebral tissues.

The effects of pentobarbital occur in much smaller concentration and,

therefore, lend support to Quastel's conception that the pharmacological actions of the barbiturates depend upon their depression of brain metabolism (7).   The profound absolute diminution of cortical respiration with pentobarbital indicates that the barbiturates may exert effects chiefly on that portion of the brain and not on the brain stem where the depression is least when reckoned on a percentage basis.   Keeser and Keeser (8) reported the greatest concentration of barbiturate in the brain stem. This and other evidence led Pick to conclude that the barbiturates act chiefly on the brain stem.   Koppanyi, Dille and Krop (9), however, found similar distributions of barbiturates throughout the various portions of the central nervous system.   If the barbiturates are thus equally distributed, then the part of the brain with the greatest oxygen requirement, namely, the cortex, would be the first to succumb to the effects of this narcotic. By the same reasoning the brain of the newly born rat with its lower metabolism might be expected to be more resistant to barbiturates than the adult brain.   It may be pointed out that recordings of the electrical potentials of the brain in dogs receiving pentobarbital reveal that the cortex is depressed long before the hypothalamus (10).

SUMMARY AND CONCLUSIONS

1. The various parts of the adult rat brain have different metabolic rates and in the following order, cerebral cortex, brain stem, cerebellum, and medulla.

2. With huge doses of alcohol and pharmacological amounts of pentobarbital the absolute depression of metabolism is greatest in the cerebral cortex.   On a percentage basis the brain stem is least affected.

3. Cerebral tissue of newly born rats is less sensitive than that of adults to alcohol and pentobarbital.

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ORIGIN AND EXTENT OF GASTRO-INTESTINAL MOTILITY IN THE CAT AND GUINEA PIG

DIRECT OBSERVATIONS ON FETUSES

R. FREDERICK BECKER AND W. F. WINDLE[1]

From the Anatomical Laboratories, Northwestern University Medical School, Chicago

It has been determined by roentgenographic technique that swallowing and defecation in amnio are normal physiologic functions in the guinea pig fetus. Swallowing of amniotic fluid and passage of material along the gastro-intestinal tract have been demonstrated in a few human fetuses. These studies have shed little light upon the nature of early propulsive movements, and the possibility of the occurrence of such motility prior to the time swallowing begins has not been considered (2, 6).

Motility of the excised fetal stomach has been described and active hunger contractions have been recorded in prematurely delivered animals. A few direct observational studies of fetal intestines in situ have been made (6, 10). The latter were based on asphyxiated fetuses and do not represent fairly the status of gastro-intestinal activity in fetuses with placental circulation functioning.

The roentgenologic studies have shown that the gastro-intestinal tract is normally active during the last third of fetal life. The present study will indicate the types of gastro-intestinal activity elicitable at various stages of gestation under the conditions of laboratory experimentation.

METHOD. Sixty cat fetuses ranging in age from 25 to 64 days (term, 65–67 days) and 51 guinea pig fetuses, 15 to 57 days old (term, 67–69 days) were used.[2] Gestation was dated from verified matings in the laboratory. Twelve of the 18 pregnant animals used were decerebrated by the anemia method (4) prior to delivering their fetuses into a constant temperature bath (37–38°C.) of Locke's solution. This made it possible to proceed without an anesthetic. Placental circulation was maintained. The remaining 6 cats (24 fetuses), threatening abortion, were anesthetized with nembutal, ether, or light ether and procain. The fetuses of aborting cats were examined at room temperature on the lab-

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[2] About 30 additional cat fetuses were studied. The results comprise a preliminary report (8).

oratory table. In two such animals, the placentas were disengaged from the uteri before the fetal intestines were exposed.

The majority (44) of the guinea pig fetuses were delivered into a bath of Locke's solution (usually about 35°C.) subsequent to infiltration of the maternal abdominal walls with 1 per cent procaine. The rest were delivered after nembutal or ether anesthesia. Placental circulation was maintained in all but 2 of the 25 pregnant animals.

In the bath, the delivered fetus was floated upon a submerged platform, its limbs fastened securely by bull-dog clamps or threads and the stomach and intestine exposed through abdominal incisions. The slender intestines of the younger specimens were observed through a binocular microscope magnifying 36 times. For older fetuses, a large reading glass proved helpful. In some experiments the umbilical cord was temporarily or permanently occluded to observe the effects of asphyxia upon gastrointestinal motility. Motion picture records of typical experiments were made.

It was important to keep the fetal organs submerged. Protrusion from the bath often led to severe, localized, intestinal knotting. The temperature of the bath could be lowered several degrees without affecting intestinal motility, but one or two degrees' elevation often led to diminished tone and quiescence.

RESULTS. Cat fetuses ranged in size from 16.5 mm. to 130 mm. crown-rump length (0.5 to 91.0 grams). Until about the 35th day of gestation, the amniotic fluid was usually clear and abundant; in two instances it was straw-colored. Intestines were white and no sharp differentiation between large and small intestine was possible at this early stage. Between 35 and 40 days, the amniotic fluid varied from straw color to a deep yellow. Toward the end of this period swallowing may have begun for the small intestine likewise appeared yellow in color while the large intestine remained white. The small intestine first appeared definitely thicker than the large intestine on the 45th day, and at this time it contained an abundant soft, yellow material; the large intestine was clear and apparently empty. By the 55th day the amniotic fluid had a greenish tinge, and there was less of it than there had been earlier. The small intestine was packed with meconium. Meconium usually filled the proximal part of the large intestine although several fetuses still showed an empty colon and yellow fluid occupied the amniotic sac. In 60 to 64 day fetuses, only a cubic centimeter or less of free fluid was found. Green meconium plastered the body of the fetus. The large bowel was packed with semi-fluid meconium.

Under optimum experimental conditions, no spontaneous gastrointestinal activity was found in 25 day old cat fetuses (16.5 mm.), although the intestines contracted locally when pinched lightly. True intestinal

peristalsis was present in 28 day (23 mm.) fetuses, though it was more frequently encountered in 29 and 30 day specimens (27 to 28 mm.). Gastric peristalsis was present for the first time on the 29th day. The constrictions were deep, and moved from the middle of the stomach toward the pylorus. In the 35 to 40 day old (36 to 65 mm.) fetuses a contraction wave passed over the stomach every 15 seconds; gastric activity persisted as long as three-quarters of an hour in the water bath. Rhythmic segmentation predominated in the small intestine, although intervals of moderate peristalsis were common. Gastric peristalsis was rarely encountered and motility in the small intestine was sluggish in fetuses older than 50 to 55 days (90–110 mm.). This condition persisted until birth. The large intestine was singularly inactive at all ages as long as observations were conducted in the water bath. Nevertheless, meconium was found in the amniotic sac at least as early as the 58th day (120 mm.).

Clamping the umbilical cord led to a change in type of gastrointestinal activity even in cat fetuses as young as 35 days (36 mm.). Gastric peristalsis ceased within two minutes. If intestinal peristalsis preceded clamping, it gradually gave way to a generalized rhythmic segmentation involving the entire intestine. Usually, within 15 minutes after complete occlusion of the umbilical vessels, segmentation diminished, the gut smoothed, and writhing pendulous movements appeared; these persisted even after death of the fetus. Some festuses showed no spontaneous activity in the intestine until the cord was clamped. Then rhythmic segmentation commenced, lasted only momentarily, and within ten minutes the intestine again became quiet, although severely constricted. A normally inactive intestine could be excited to activity temporarily by lightly clamping the cord. Restoration of placental circulation led to normal quiescence within two or three minutes. Similarly, intestinal peristalsis could be abolished in favor of generalized rhythmic segmentation, only to reappear after the clamp was released. However, when the intestines were active, permanent obliteration of the umbilical circulation always finally led to pendulous, agonal writhing.

Deep ether anesthesia administered to the pregnant mother before delivery rendered the fetal gastro-intestinal tract entirely unresponsive even to mechanical stimulation. When the fetuses were quickly removed after light ether anesthesia and local procaine, the intestines responded with immediate hyperactivity just as they did when the umbilical cord was first clamped. Within 15 minutes, a diminution of intestinal tonus ensued. Nembutal anesthesia, on the other hand, led to a general depression of all fetal activity, lasting as long as 30 minutes in some cases. A period of hyperactivity followed; gastric peristalsis, rhythmic segmentation and occasional intestinal peristalsis seemed brisker than that normally encountered. These effects were noted in specimens as young as 33 days'

gestation (35 mm.). When a general anesthetic had been administered, anastalsis and nondirectional propagation were encountered part of the time in the fetuses younger than 45 days old (75 mm.). Gastric peristalsis often suddenly reversed and proceeded toward the cardia. In the intestine, peristaltic waves traveled both orally and aborally from a constriction. Sometimes the direction of propagation suddenly reversed in several loops of intestine while it continued aborally in others.

Guinea pig fetuses ranged from 5 mm. to 120 mm. (0.15 to 87 grams) crown-rump length. The amniotic fluid was colorless until about the 42nd day of gestation. By the 36th day, the cecum clearly marked the division between small and large intestines although it had not yet become sacculated. By the 50th day, the small intestine was light yellow in color and the amniotic fluid was a darker yellow. The cecum and proximal colon were white and sacculated as far as the descending colon. The latter was colored a deep yellow to brown and had a knotted appearance although its contents were soft. Between 55 and 60 days, the amniotic fluid contained particles of light yellow meconium. Between 60 and 67 days, the fluid was sparse and contained a deep yellow or green colored meconium. The small intestine was usually empty and inactive during this period of development, but the large intestine was distended and, at 60 days, was packed with a soft yellow mass. Near term, meconium of a more solid nature than heretofore appeared in knot-like constrictions along the descending colon.

No gastro-intestinal motility was observed in the fetal guinea pig until the 27th day (17 mm.). At this time local contractions appeared when the intestine was pinched lightly. Rhythmic segmentations of short duration were noted on the 29th day (22 mm.) during asphyxia (placentas of these fetuses had been separated from the uterus). True gastric and intestinal peristalsis was not encountered until the 35th day (35 mm.) and was sluggish. By 40 days (48 mm.) peristalsis was seen in both the large and small intestine, more frequently in the latter. The cecum contracted rhythmically in 42 day (50 mm.) specimens. Gastric peristalsis was still sluggish and arrhythmic. It became more coördinated and rapid by the 45th day (67 mm.). At 50 to 55 days (80 to 95 mm.) peristalsis was found mainly in the ascending colon. When it occurred with localized rhythmic segmentation of the small intestine, it carried the intestinal bolus for a distance of 8 to 10 mm. In the proximal colon, the bolus was sometimes moved as much as 2 cm. so rapidly that it simulated a peristaltic rush. Usually, however, the proximal colon appeared tonically constricted and blanched but was otherwise inactive. Gastric peristalsis, when present, was about as active as in the newborn. Contraction waves passed from the middle of the corpus over the pyloric antrum at the rate

of 1 every 3 seconds. Most activity in 60 to 67 day (110–120 mm.) fetuses was restricted to the large intestine. Peristaltic rushes were frequent, carrying the contents from cecum to descending colon. Segmentation was prominent in the proximal colon. Activity was rarely seen in the small intestine. Gastric peristalsis, when observed, was always brisk.

The gastro-intestinal tract of the fetal guinea pig responded to experimentally induced asphyxia and to general anesthesia exactly as it did in the fetal cat.

COMMENT. The fact that the intestinal musculature of both the cat and guinea pig fetus responded to light pinching before spontaneous activity was present agrees with Yanase's observations (10) on freshly killed guinea pig fetuses 15 mm. long. He reported that peristalsis appeared in the 19 mm. guinea pig, but it is not clear that he meant propagational movements. Under the best of our experimental conditions in the water bath with placental circulation intact we were unable to observe true peristalsis in the guinea pig until the 35th day (35 mm.). Rhythmic segmentation did appear in younger specimens but only under poor conditions (impaired placental circulation).

Apparently gastro-intestinal activity begins later and at a lower level of activity in the fetal guinea pig than in the cat. From the 50th day to term, however, gastro-intestinal motility functioned at a more mature level than in the fetal cat of comparable age. Species differences in motility may have been due to structural differences in the cecum and colon. In the cat during the last quarter of gestation the simple, tube-like cecum and colon were distended with meconium, peristalsis was infrequent, and rhythmic segmentation seemed restricted to the small intestine. Apparently defecation in amnio is not as common in the cat as in the guinea pig (2). Perhaps distention of the lower bowel acted reflexly in the cat fetus to inhibit motility in the stomach and proximal intestine. In the guinea pig, on the other hand, the cecum and proximal colon were large, sacculated, and active. In specimens 55 days or older the small intestine was often empty, and mass peristalsis was commonly seen in the large bowel. That defecation and reswallowing of fluid may occur as often as five times before birth has been reported (2).

Motility of the fetal gastro-intestinal tract seems to follow a definite developmental pattern. The earliest activity encountered was strictly localized. It consisted of contraction and relaxation following mechanical or electrical stimulation of the gut. Except for its slowness of execution, it was much like the earliest localized somatic movements of the mammalian embryo (5, 7, 9). Whether a local rhythmic segmentation or a true peristalsis is the next step in development was impossible to determine with certainty. Under the best of conditions in 9 of the 15 early cat

embryos (28–30 days old) peristalsis was observed in intestines which showed no localized rhythmic segmentation. In the remaining 6 embryos studied in air or with placentas detached, segmentation predominated but there were sporadic periods of peristaltic activity. These changes were attributable partly to asphyxia. In 3 of 5 early guinea pig fetuses (35–36 days old) peristalsis was present as soon as the intestines were exposed, but within twenty minutes rhythmic segmentation also appeared. Even before this, rhythmic segmentation alone was elicited by asphyxia in three fetuses (29 days). Often it was possible in older specimens to observe both phenomena in the same intestine. However, neither rhythmic segmentation nor peristalsis, complex patterns of behavior, appeared to be established until several days after the gut was able to respond locally.

TABLE 1

Summary of events in the development of G.-I. motility

	CAT		GUINEA PIG	
	Days	Millimeters	Days	Millimeters
1. Marked vagal innervation of stomach and sympathetico-vagal innervation of intestines..................................	23	13		
2. Enteric plexuses and smooth muscle present. Local contraction of intestine to pinching.................................	25	16.5	27	17
3. Rhythmic intestinal segmentation induced by anoxemia.....................	28	23	29	23
4. True intestinal peristalsis began.........	28	23	35	35
5. True gastric peristalsis began............	29	27	35	35
6. Colonic peristalsis began................	42	70	40	48
7. Defecation began.......................	58	120	55–60	95–110

In a preliminary report, Windle and Bishop (8) offered an explanation for the changes in gastro-intestinal behavior which ensue during anoxemia. They correlated changes in the oxygen content of umbilical vein blood with changes in motility. The present results are in accord with these. Similar changes in fetal somatic motor responses during anoxemia have been described (7). Other examples of smooth muscle activation during partial anoxemia (at birth) may be the constriction of the ductus arteriosus sphincter and the contraction of the fetal spleen (6).

Burstein (3) has recently examined the effects of the barbiturates, including nembutal, upon intestinal activity in adult dogs with Thiry-Vella loops of the upper jejunum. Balloon tracings recorded an immediate depression of rhythmic intestinal contraction and a fall in tonus lasting

from 5 to 15 minutes. The primary effect was transient and was succeeded by a more prolonged phase of heightened intestinal contraction and tonus. Evidently, the fetal intestinal musculature responded like the adult in this respect.

Alvarez (1) has reported that stimulation of the small intestine of the rabbit at any point gives rise to a wave of contraction which travels both orally and aborally. A similar condition was encountered in several of the fetal cats and guinea pigs early in gestation; this reaction was spontaneous. Whether such behavior is really a refutation of the "law of the intestine" or not is difficult to say; in our experiments it occurred only under duress of general anesthesia.

Although histological study of the gastro-intestinal tracts of the fetuses is still incomplete, a few interesting facts have been ascertained. By the time the fetal gastro-intestinal tract of the cat and guinea pig begins to exhibit peristalsis, both circular and longitudinal muscle are present in the stomach and intestine. The myenteric and submucous ganglia and plexuses are present for several days before gastro-intestinal motility begins. In fact they are present before local constrictions are first elicitable. In 13 mm. to 18 mm. cat embryos, a rich vagal supply can be traced to the stomach and a prominent sympathetico-vagal supply leaves the coeliac ganglia for distribution to the intestinal tract.

SUMMARY

1. The earliest indication of motility in the gastro-intestinal tract of both the fetal cat and guinea pig was a simple localized contraction in response to stimulation (25–27 days, approximately 17 mm.).

2. Spontaneous gastric and intestinal peristalsis was present in cat fetuses of 29 to 30 days' gestation (27–28 mm.) and in guinea pig fetuses on the 35th day (35 mm.).

3. Peristalsis was observed earlier than rhythmic segmentation in the cat, but both types of motility often were present in the same intestine. Segmentation was elicitable before spontaneous peristalsis appeared in the guinea pig.

4. Peristalsis became progressively more active in the stomach and small intestine up to about 50 days' gestation. Thereafter it became less active in the small intestine of both species. In the guinea pig, however, the stomach, cecum and colon retained a high degree of motility throughout.

5. After clamping the umbilical cord, peristalsis gave way to generalized rhythmic segmentation and then to diminished intestinal tonus with slow, pendulous writhing of the intestine. Anoxemia likewise excited inactive intestines to an initial hyperactive state of generalized rhythmic contrac-

tion and relaxation; soon activity ceased, but the intestines remained severely contracted for some time.

6. Light ether anesthesia had an effect upon the fetal intestine like that engendered by clamping the cord. Deep ether anesthesia inhibited activity completely.

7. Nembutal administered to the pregnant mother first depressed fetal gastro-intestinal activity; gradually tonus and rhythmic motility returned and finally the gut became hyperactive.

8. Anastalsis and general nondirectional activity were characteristics of the hyperactive state during anesthesia in young fetuses of both species.

9. By the time peristalsis was established in the fetal cat and guinea pig, circular and longitudinal muscle surrounded the gastro-intestinal tract.

10. The myenteric and submucous ganglia and plexuses were established as early as 17 mm. in both species, while a rich vagal supply to the stomach and an equally prominent sympathetico-vagal supply from the coeliac ganglion to the intestinal tract were present in the cat at 13 mm.

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CERTAIN QUANTITATIVE ASPECTS OF THE PANCREATIC RESPONSE TO SECRETIN

HARRY GREENGARD, IRVING F. STEIN, Jr. AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

In the very large number of secretin assays performed in this laboratory according to the method described by us (1), we have repeatedly observed that there was no direct proportionality between the amount of secretin injected and of the resultant output of pancreatic juice. Thus, it has been noted that doubling a small dose of secretin will approximately treble the secretory response. It has also been our impression that the reaction to injections of isolated quantities of secretin does not resemble the condition existing when the pancreas secretes in response to a meal. There is no information at present regarding the minimum quantity of secretin which must be present in the circulation in order a, to cause the pancreas to secrete at a minimal and constant rate, and b, to cause the gland to secrete at its maximal rate.

The following experiments have been performed with a view to clarifying these problems.

EXPERIMENTAL. Intact dogs were anesthetized with sodium pentobarbital, the femoral vein exposed, and the main pancreatic duct cannulated in the usual manner. The secretin used was our SI preparation, 0.25 mgm. of which stimulates the pancreas of over 50 per cent of dogs to secrete 10 drops when injected intravenously, or represents a dog unit (1) or "threshold dose." The volume response of the pancreas to graded doses of the secretin was measured by collecting the pancreatic flow in a small graduated cylinder and noting the volume obtained during the first 10 minutes after injection and also for the entire duration of action. The animals were always permitted to return to the basal level after each stimulation of the pancreas. Injections were made at a rapid rate.

A second series of dogs was prepared in a similar manner, except that secretin solutions of graded concentration were injected into the femoral vein at a constant rate by a Woodyatt pump. The secretory response was measured in drops.

RESULTS. *Threshold dose of secretin.* The usual individual variations were noted in the 25 dogs used in these experiments. The threshold dose

was 0.2 mgm. in two dogs, 0.25 in twelve, 0.5 in six, 1.0 in four, and 1.5 in one. The percentage distribution of the animals is shown in figure 1.

Effect of single secretin doses in increasing amounts. In the 25 animals studied there was uniformly a sharp increase in the quantity of flow in response to increasing secretin dosage. With further secretin additions the secretory increase became progressively less marked and finally a
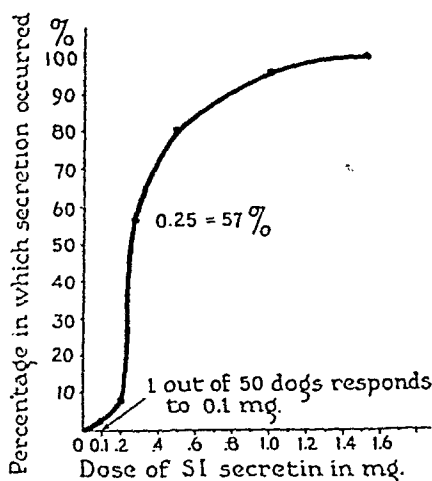
Fig. 1. Distribution curve in 25 dogs showing individual variations in threshold dose of secretin.

TABLE 1

INJECTION (THRESHOLD DOSES)	SECRETORY RESPONSE		Δ		DURATION OF ACTION
	1st 10 min.	Total	1st 10 min.	Total	
	cc.	*cc.*	*cc.*	*cc.*	*minutes*
1	0.4	0.4	0.4	0.4	10
2	1.2	1.4	0.8	1.0	20
3	1.9	2.3	0.7	0.9	25
4	2.5	2.9	0.6	0.6	29
5	3.1	3.4	0.6	0.5	32
6	3.4	3.9	0.3	0.5	35
8	3.7	4.5	0.15	0.3	34
10	4.2	5.2	0.25	0.35	33
12	4.3	5.6	0.05	0.2	33
14	4.4	6.0	0.05	0.2	35
16	4.6	5.9	0.1	−0.05	33

secretin dosage was obtained which stimulated the pancreas to its maximum capacity. Beyond this point further increases in secretin administered elicited a fairly constant response. The average responses in the 25 animals are listed in table 1. In this table are summarized the secretory responses for the first 10 minutes, the total response, the duration of action of the secretin, and the value designated by us as Δ, or the increase in secretion per unit increase in secretin threshold doses.

Effect of continuous injection of secretin in increasing amounts. This technique was carried out on 5 dogs. The results are tabulated in table 2.

DISCUSSION. The individual variations in threshold dosage of our SI preparation were of the same order as those previously observed by us in very many animals. Since this information has never been published and since it was available on the 25 dogs studied in this work, we are including it in the present report.

It is evident from the findings tabulated above that no simple proportionality exists between the magnitude of a given dose of secretin and the pancreatic response. In all cases the effect of increasing single doses of secretin was an initial sharp increase in pancreatic secretion, followed by a gradually lessening increase in response until a maximum flow was obtained which could not be exceeded by any further increase in administered secretin. The average responses for the 25 dogs tested are represented in figure 2. The relationship between dosage and degree of response is a

TABLE 2

SECRETIN THRESHOLD DOSES PER MINUTE	RESPONSE IN CC. PER 15 MINUTES				
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
0.05					0.3
0.10	1.5	1.0	0.8	1.0	0.6
0.20	5.6	2.4	1.0	1.5	2.7
0.30				2.5	5.7
0.40	5.7	9.0	2.3 .	3.1	9.0
0.60		12.8	5.0	4.1	13.0
0.80		11.2	6.2	5.0	15.2
1.00			7.5	4.3	15.9

curvilinear one in which the curve assumes an S-shape. The same type of curve is obtained both for the 10-minute and the total responses; the former reached its maximum value at a lower dosage level than did the latter. It was also noted that although, as expected, the action was more prolonged in higher than in lower dosages, the duration of action reached a maximum before the total flow did so.

The S-shaped curve (fig. 1) denoting the variation in the threshold dose in different animals is to be expected (2). The concentration-action curve (fig. 2) is also S-shaped. This might also be expected since when a minimal effective amount of secretin is injected the response is small, and when the dosage of secretin is progressively increased above this minimal one the pancreatic output is elevated to a degree out of proportion to the increase in dosage. Thus when a curve is plotted with secretin dosage as the abscissa and pancreatic response as the ordinate there is at first a gradual, then a sharper rise in response; and finally the rise again becomes gradual

and levels off when a secretin dosage is attained which stimulates the gland to secrete at its maximum capacity.

These results demonstrate that it is fallacious to assay unknown secretin preparations by administering sizable amounts to an animal and interpreting the dosage by calculation on the basis of direct proportionality in terms of a very small and arbitrarily fixed response (3).

The experiments in which graded doses of secretin were injected continuously at uniform rates yielded results consistent with those obtained by single injections in that as the quantity of secretin injected per unit of time was increased there was at first a rapid, then a more gradual increase in volume output of secretion. The minimum effective dose found was 0.05 threshold dose per minute and a maximal flow was obtained at 1 threshold dose per minute. Since in previous experiments it has been noted by us (1) that the threshold dose of pure secretin base in the dog is
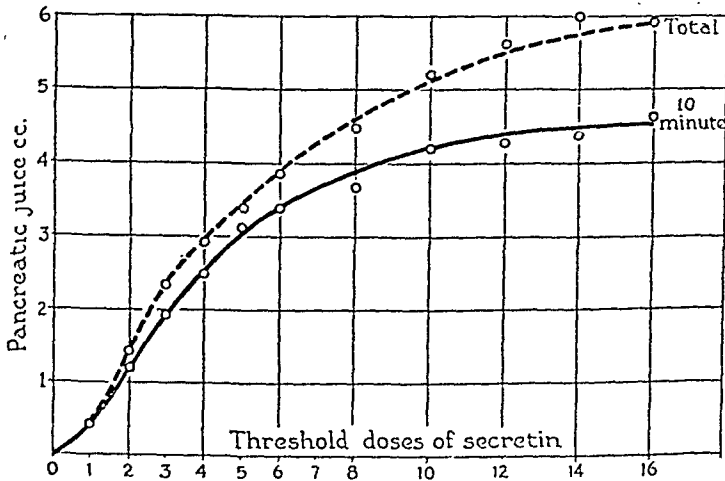
Fig. 2. Total and 10-minute volume output of pancreatic juice in response to increasing doses of secretin.

0.014 mgm., it follows that the minimum amount necessary to be present in the circulation of an *anesthetized* dog is 0.7 gamma, and the amount required to cause the gland to secrete at its maximum rate is 14 gamma. These statements are based on the assumption that when secretin is injected at a constant rate a condition of equilibrium is attained in the animal so that the rate of "destruction" equals the rate of injection and a constant amount of secretin is present in the circulation for any given continuous dosage less than that quantity which exerts a maximal effect. The constant secretory rates which we have found appear to bear out this assumption, the doses of secretin used being less than supramaximal.

SUMMARY AND CONCLUSIONS

The volume response of the pancreas to graded doses of secretin has been studied. When secretin is given in single doses in increasing amounts

the pancreatic flow increases; this increase takes place rapidly when the dosage is small and gradually when it is large. A dosage is finally obtained which causes the gland to secrete at its maximum capacity and beyond which further increases in the quantity of secretin injected give no greater response. The increase in volume output when measured for the first 10 minutes was similar to that noted for the total duration of action. Any assay method based on the assumption that a direct proportionality exists between the amount of secretin injected and the amount of juice secreted is fallacious, since the relation is curvilinear. When secretin was given continuously in increasing amounts, the results were analogous to those obtained with single doses. It is concluded from the continuous injection experiments that the minimal effective amount of pure secretin base necessary in the circulation of the average anesthetized dog is 0.0007 mg. and the amount requisite to stimulate the gland to secrete at its maximum capacity is 0.014 mgm.

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INFRARED SPECTROPHOTOMETRIC STUDIES ON HEMO-GLOBIN AS AFFECTED BY CYANIDE, METHYLENE BLUE AND CARBON MONOXIDE

MATILDA MOLDENHAUER BROOKS

From the University of California, Berkeley

The purpose of this investigation was to find out by means of absorption spectra whether any change in the atomic configuration of the hemoglobin molecule in the infrared region of the spectrum takes place when various reagents are added. This information will be valuable in further cor-. roboration of interpretations concerning the mechanism of methylene blue and cyanide when added to blood.

Considerable work has been done in the visible and ultraviolet portion of the spectrum but very little in the infrared part. The only other experiments known to the writer on near-infrared absorption bands of hemoglobin are those of Hartridge and Hill (1914), who found an absorption maximum of oxyhemoglobin at about 9000 Å, and those of Eggert (1935). In the measurements of the writer, no difference between the absorption of hemoglobin and that of water around 9000 Å was found.

METHODS. The experiments[1, 2] given in this paper were done with a Bausch and Lomb spectrophotometer with camera attachment and absorption spectra photographed on Eastman infrared photographic plates.

Curves as shown in the figures were obtained from these spectra using a microphotometer.

In these experiments a 20 per cent solution of sheep blood dissolved in 0.4 per cent NH_4OH or H_2O was used. Eastman spectroscopic plates nos. P, Q, R and Z, hypersensitized in 20 per cent NH_4OH immediately before use, and panchromatic plates were used. Each of these plates is sensitive over a specified range of the spectrum from about 6500 Å to 12,000 Å as shown in table 1.

The various derivatives of hemoglobin solutions were produced as follows:[3] To 2 cc. of blood was added one of the following: (1) KCN; 0.2 cc. of 0.02 M. (2) $NaNO_2$; 1.00 cc. of 0.2 per cent diluted in NaCl. (3) Methylene blue; 0.1 cc. of 0.1 per cent. (4) $K_3Fe(CN)_6$; 0.5 cc. of 3.0 per cent. (5) Glucose; 4.0 cc. of 5 per cent.

The final dilution was brought up to 10 cc. by adding water or 0.4 per cent NH_4OH solution in each case.

TABLE 1

NUMBER OF PLATE	WAVE LENGTH IN Å—MAXIMUM SENSITIVITY
	Visible spectrum and red up to:
Panchromatic.......................	6,800 Å or 7,000 Å
R Plate............................	7,600 to 8,400 Å
P Plate............................	8,000 to 9,000 Å
Q Plate............................	9,400 to 10,300 Å
Z Plate............................	10,900 to 12,000 Å

Various combinations of these solutions, using the given proportions were also made, such as blood, KCN and methylene blue; blood, $NaNO_2$ and KCN; and so on as indicated in the graphs. The exposure of the plates varied with the kind of plate used. With the visible plates a 20 per cent solution of blood was usually used at a depth of 1 cm. in the spectrophotometer for 3 to 10 seconds. With plates of longer wave lengths, a 10 per cent solution was used and the exposure in some cases was 1 hour. The tube containing the blood was cooled with a fan. Since each series of plates varied, tests were made to give exposures which would be resolved by the microphotometer into curves with sharp maxima.

Inasmuch as the photographic plates represent degrees of blackening, or transmitted light, the results are given as percent transmission, which is the reciprocal of absorption. The height of the curves is significant in the plates which represent the infrared region (P, R, Q and Z) because com-

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[3] In a previous paper equivalent concentrations of reagents were added to blood (Brooks, 1935). In this paper smaller concentrations of KCN were used to approximate more nearly the conditions found in cyanide poisoning.

parable exposures could be used. In the case of the panchromatic plates which represented the visible portion of the spectrum, the light solutions could not be given the same exposure as the dark ones without producing total blackness. In these plates, therefore, the heights of the curves of solutions containing methemoglobin are not comparable with those for hemoglobin, whereas "light source" curves were given the same exposure as "hemoglobin" curves. The chief object of this paper is to show shifts in the maxima rather than density differences, so that heights of curves are not important here.

As to the dispersion comparisons, in the infrared region the spectrophotometer was adjusted to produce maximum effects. This would show differences when comparing calibration lines in different plates, but would not alter the position of absorption or transmission maxima.

Controls of the reagents alone without blood, of the light source for each plate alone, and of water, were also made. The "light source" curve represents the sensitivity of the plate itself which was determined for each plate. Calibrations of the curves with line spectra of the neon, argon or mercury arcs were also obtained according to wave length desired. Deviations in the transmission maxima from those of the appropriate controls were attributed to the reagents added to the experimental solution. When the proper density of the band spectra had been obtained, these were microphotometered and the curves so obtained are those represented in the various figures discussed below.

Abbreviations of the various solutions were as follows: CN for KCN; NO_2 for $NaNO_2$; MB for methylene blue; Hb for blood in the form of oxyhemoglobin; $FeCN_6$ for $K_3Fe(CN)_6$; "Source" for light source without any solutions.

RESULTS. Figure 1 shows similarity of the transmission curves for source, water and blood using a Q plate which has a maximum transmission around 10,000 Å. Curves for various dilutions of blood are similar.

Panchromatic plates. These plates are sensitive from the region of visible light up to slightly over 7000 Å. Figure 2 shows the source (curve 1), that for Hb alone (curve 2), and that for Hb + NO_2 (curve 3). In the latter, the hemoglobin is converted to methemoglobin and shows the shift in the bands towards the red end. Figure 3 shows the difference between the maximum transmission for blood when CN and MB were added (curve 1) as compared with that for blood when CN and NO_2 were added (curve 2). The addition of CN in this concentration to methemoglobin (curve 2) did not produce any shift in the maximum, owing to the relatively greater concentration of $NaNO_2$ present. Nor was there any indication of an additional band such as would be expected if KCN united with methemoglobin to form a new compound. Nor was there any shift in the maximum when CN was added to blood containing MB (curve 1). Figures 2 and 3

Abscissae represent wave lengths in Å: ordinates represent per cent transmission

Figs. 1–9

were exposed on the same spectroscopic plate but on different micro-photometer plates, using different ordinate scales.

Figures 4 and 5 are from the same plate, which is sensitive only to 6900 Å. Figure 4 shows that the maximum for blood and MB (curve 1) is the same as that for blood to which CN and MB had been added (curve 2), while a shift occurs in the curve for methemoglobin (curve 3) produced by adding NO_2 to blood which has stood long enough to consume the reductants. Figure 5 shows further combinations of blood, NO_2, CN and MB (curve 2) in which the maximum had again shifted to about 6590 Å from 6402 Å, as compared with curve 3 which is for blood with CN and MB added. Here again the formation of methemoglobin when NO_2 was present is to be compared with the absence of methemoglobin in spite of the combination of CN and MB.

These curves show that methemoglobin shifts the transmission maximum towards the infrared region; that blood containing methylene blue either alone or with cyanide does not cause this shift; whereas cyanide and NO_2 together in those concentrations produce no shift in the methemoglobin curve.

P-plate. This plate has a maximum sensitivity around wave length 8424 Å. Figure 6 gives the curve for source (curve 1); Hb (curve 3); and Hb and CN (curve 2). The maxima are all alike, with the primary at about 8400 Å and the secondary at about 7100 Å. The Hb curves are

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Fig. 1. Showing similarity of the curves for source (curve 1), water (curve 2) and blood (curve 3). (I-Q plate #65,—1, 2, 3.)

Fig. 2. Showing difference in the curves for blood and $NaNO_2$ (curve 3) as compared with source (curve 1) and blood (curve 2). (Pan. plate #83,—1, 2, 3.)

Fig. 3. Showing difference in the curves for blood containing cyanide and methylene blue (curve 1) as compared with blood, cyanide and $NaNO_2$ (curve 2). (Pan. plate #83—4, 5.)

Fig. 4. Showing the effect of $NaNO_2$ and methylene blue on blood (which has stood) (curve 3), as compared with the lack of effect with cyanide and methylene blue (curves 1 and 2). (Pan. plate #38,—1, 3, 5).

Fig. 5. Showing the absence of effect of added cyanide and methylene blue to blood (curve 3) as compared with cyanide, $NaNO_2$ and methylene blue (curve 2). Curve 3 is of methylene blue. (Pan. plate #38.)

Fig. 6. Showing similarity of the curves for source alone (curve 1), blood (curve 3) and blood containing cyanide (curve 2). (I-P plate #67,—1, 2, 3.)

Fig. 7. Showing difference in the curves for blood containing $NaNO_2$ (curve 3) as compared with blood containing cyanide and methylene blue (curve 2) and the similarity between curve 2 and curve 1, which is of blood and cyanide. (I-P plate #67,—3, 4, 5.)

Fig. 8. Showing the influence of methylene blue on fresh blood containing $NaNO_2$ (curve 2) as compared with that on blood and methylene blue (curve 1). (I-P plate #67,—7, 8.

Fig. 9. Showing similarity in blood oxidized by either $NaNO_2$ (curve 1) or potassium ferricyanide (curve 2). (I-P plate #67.)
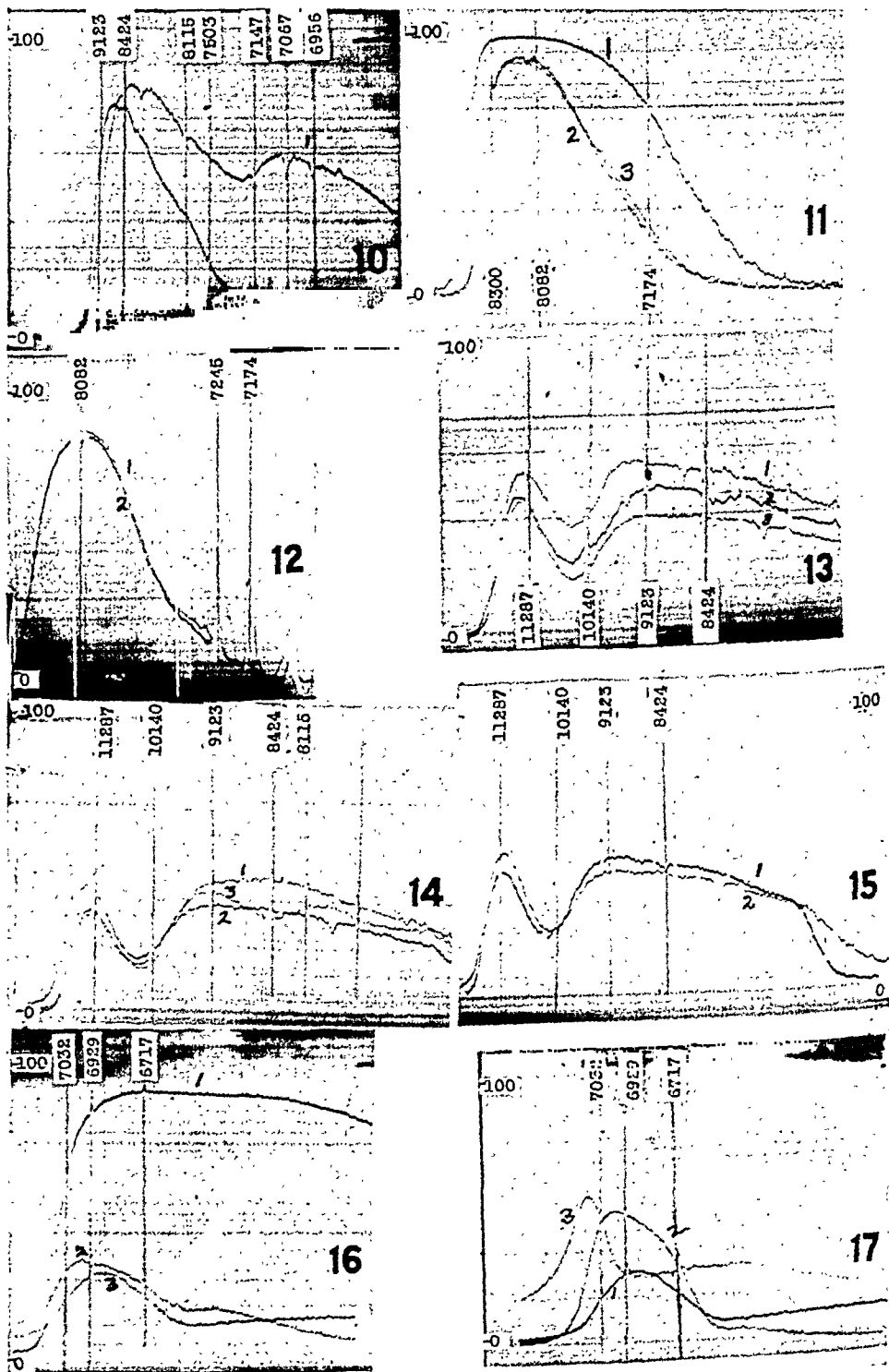
Abscissae represent wave lengths in Å; ordinates represent per cent transmission

Figs. 10–17

slightly lower than the curve for the plate alone.   Figure 7 shows the effects of NO_2 upon the primary transmission (curve 3).   The maximum is shifted toward the infrared end to about 8500 Å, whereas the curve for blood containing CN and MB has the same maximum as that for blood and CN alone.   The changes in the secondary maximum in the visible region due to the blue color of methylene blue are better shown elsewhere.   Figure 8 shows the influence of MB on fresh blood containing NO_2 (curve 2) as compared with Hb and MB alone (curve 1).   The maximum of curve 2 is only slightly shifted to the left.   This can readily be seen when compared with the curve for methemoglobin of figure 7 (curve 3), all of which were photographed on the same plate.   Figure 9 shows the curves for methemoglobin produced by ferricyanide (curve 1) or by NO_2 (curve 2).   They are essentially identical with the primary maximum at about 8500 Å and the secondary at about 7100 Å.   Figure 10 shows more plainly the shift in the maximum when pure methemoglobin produced by ferricyanide was used (curve 2) as compared with the source (curve 1).

These curves show conclusively that MB does not produce methemoglobin in the blood when CN is present.

R-plates.   The sensitive region of this plate is around 8082 Å as shown by curve 1 of figure 11.   There is no shift in the maximum with any of the solutions used, as shown in figure 11 and figure 12 for Hb, Hb and NO_2, and Hb and CN.   Other experiments not here represented for water, NH_4OH or MB and in combinations, give similar results.

Z-plates.   The region from 12,000 Å down to 8100 Å is the sensitive part of the Z-plates.   There is no difference from the controls in the maximum of any of the solutions used from 11,287 Å to 9123 Å, as shown in

Fig. 10. Showing difference in the curves for blood containing K ferricyanide (curve 2) as compared with source (curve 1).   (I-P plate #67,—1, 6.)

Fig. 11. Showing similarity in the curves for source (curve 1), blood (curve 2) and blood containing $NaNO_2$ (curve 3).   (R-plate #81,—1, 2, 3.)

Fig. 12. Showing similarity in the curves for blood (curve 1) and blood with cyanide (curve 2).   (R-plate #54.)

Fig. 13. Showing similarity in the curves for source (curve 1), NH_4OH (curve 2) and blood (curve 3).   (I-Z plate #69,—1, 2, 3.)

Fig. 14. Showing difference in the curves for blood and $NaNO_2$ (curve 3) as compared with blood (curve 2) and blood containing cyanide (curve 1).   (I-Z plate #69,—3, 4, 5.)

Fig. 15. Showing similarity in the curves for blood (curve 1) and blood containing methylene blue (curve 2).   (I-Z plate #69,—3, 7.)

Fig. 16. Showing the sensitivity of the method in which 5 per cent methemoglobin had been added to blood (curve 2) as compared with curves for oxyhemoglobin (curve 3) and source (curve 1).   (Pan. plate #85,—1, 3, 5.)

Fig. 17. Showing the sensitivity of the method.   Curve 1, blood; curve 2, hemoglobin + 5 per cent methemoglobin; curve 3, 100 per cent methemoglobin.   (Pan. plate #85,—3, 6, 9.)

figure 13, which includes curves for the source (curve 1), NH_4OH (curve 2) and blood (curve 3). Figure 14 shows that blood containing NO_2 (curve 3) has a maximum at 9123 Å and differs also from that of Hb alone (curve 2) around 8400 Å. This is shown better on the P-plates, and on the panchromatic plates towards the violet end. Curve 1 (Hb + CN) has the same type of curve as that for Hb alone. Figure 15 shows curves for Hb (curve 1) and for Hb and MB (curve 2). Curve 2 should be slightly shifted to the right so that the calibration line 11,287 Å coincides with the same line on curve 1. This error was made in making the microphotograph. The two curves are essentially similar in this region of the spectrum and show no such differences as are found with NO_2. This again shows that methylene blue does not produce methemoglobin in the blood under these conditions.

Figures 16 and 17 are controls with panchromatic plates to indicate the sensitivity of the method. Figure 16 shows the shift in the maximum towards the red end when 5 per cent methemoglobin was added to a solution of oxyhemoglobin (curve 2) as compared with the source (curve 1) and that for oxyhemoglobin (curve 3). Figure 17 shows the difference between a solution containing 5 per cent methemoglobin in 95 per cent oxyhemoglobin (curve 2) and oxyhemoglobin (curve 1) and methemoglobin (curve 3). Methemoglobin was prepared in the usual way ($NaNO_2$) and added to oxyhemoglobin immediately before taking the picture so that minimum reduction of the methemoglobin occurred. These curves show appreciable differences.

DISCUSSION. These experiments show that in certain regions of the infrared spectrum there is a shift in the transmission maximum of blood towards the longer wave length when $NaNO_2$ is added alone or in combination, but that other reagents used here produce no shift. This confirms the writer's previous conclusions from studies of blood in the visible spectrum, indicating that neither cyanide nor methylene blue produces any changes in the prosthetic group of the hemoglobin molecule under the conditions used.

The spectrophotometric measurements and other experiments made by the writer (M. M. Brooks, 1932, 1933, 1934, 1935a, 1935b, 1939) have demonstrated that *methylene blue does not produce methemoglobin in the blood of living animals, but on the contrary changes methemoglobin to oxyhemoglobin* (M. M. Brooks, 1935, p. 170).[4] Since there is no evidence of the presence of methemoglobin formation by methylene blue when used in cyanide poisoning, this mechanism cannot be used in interpreting its

[4] Recently Wendel (1937) has confirmed the writer's experiments, reversing his opinion of 1933 and 1934, and Hartman, Perley and Barnett (1938) have also confirmed that methylene blue not only prevents the formation of methemoglobin but reduces it after it is formed.

action.   The presence of glucose and other reductants in the blood stream of living animals is an important factor in preventing the formation of methemoglobin.   This has been previously discussed by Brooks (1940) as due, in part, to the lowered redox potential produced by these substances.

Warburg (1931) states that the poisoning effect of cyanide is upon the respiratory enzyme "inactivating" it.   From the experiments of the writer it is deduced that "inactivation" means a change in the electrode potential of the system involved to a negative value where Fe^{++} of the prosthetic group remains in the bivalent form and cannot shift back to the trivalent form; and furthermore, that "recovery" means a shift in the potential back to the normal range when substances are added whose systems have suitable potentials so that the system $Fe^{++} \rightarrow Fe^{+++}$ again becomes appreciably reversible (Brooks, 1940a).   Such substances are methylene blue, $NaNO_2$, or any other nontoxic oxidant.   The formation of methemoglobin by $NaNO_2$ is merely a by-product as in the case of sulfanilamide therapy (Brooks, 1940b).   The effect of cyanide is upon the oxidation-reduction potential of the respiratory enzyme.

It is suggested from these experiments that recovery from cyanide poisoning is limited to effects upon the respiratory enzyme without invoking the methemoglobin[5] theory.   Recovery from cyanide poisoning is merely a shift back to normal in the electrode potential of the respiratory enzyme system, produced by either methylene blue or $NaNO_2$ or any other similar weak, nontoxic oxidant (Brooks, 1940a).

These experiments were done to explain the mechanism of poisoning and recovery in animals by cyanide and to show that the objections to the use of methylene blue therapy[6] in carbon monoxide poisoning are not justified.

SUMMARY

Infrared spectrophotometric and microphotometric curves of blood as affected by cyanide, $NaNO_2$, or methylene blue in the presence or absence of reductants are given:

a. $NaNO_2$ produces a shift in the transmission maximum from 6402 Å to 6590 Å and from about 8400 Å to about 8500 Å.

b. Methylene blue does not produce any shift unless the reductants are used up.

c. KCN does not produce any shift either with oxyhemoglobin or with methemoglobin, in the concentrations used.

[5] Barron, 1929; Barron and Harrop, 1930; Barron and Hoffman, 1930; Anson and Mirsky, 1932; Drinker, 1938; Harrop and Barron, 1928; Henderson, 1938; Wendel, 1933; Hug, 1933.

[6] Y. Henderson, Adventures in respiration (1938); Cecil K. Drinker, Carbon monoxide asphyxia (1938).

Therefore it is again corroborated that methylene blue does not produce methemoglobin in fresh blood. In the light of the new findings and deductions it is concluded that cyanide does not attach to the prosthetic group of either ferrous or ferric hemoglobin, but acts rather by stabilizing the electrode potential of the $Fe^{++} \rightarrow Fe^{+++}$ system of the respiratory enzyme at a negative value where it must remain in the ferrous form and cannot shift back to the ferric state.

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CORONARY REFLEX DILATATIONS ACCOMPANYING CONTRACTIONS OF VOLUNTARY MUSCLES

CHARLES W. GREENE

From the Departments of Physiology, University of Missouri and Stanford University

Physiological research literature presents an ever increasing number of observations demonstrating the close coördination of events occurring in widely separated regions of the body. We add to these lists evidence of this type of associated reactions in the skeletal neuromuscular system integrated with the autonomic nervous control regulating the flow of blood through the coronary vessels. The demonstration is the more significant in that the coördinated reactions occur in animals deprived by ether of the conscious and orderly control of the skeletal musculature.

Dogs served as experimental animals. In several hundred coronary tests of which those reported in this group are a part, the effort has been to maintain the viability of the animal in all of its complexity restricted only by the necessary use of anesthetics. By this technic one may evaluate both the passive mechanical and the active reflex factors influencing the coronary system. Ether was supplemented at times by certain hypnotics and narcotics. It is necessary to maintain a regular and strong heart beat with a vigorous blood pressure if one is to obtain orderly contractions of skeletal muscles.

If etherization is guardedly reduced to a light level it is possible, long before consciousness occurs, to secure reflex and more or less regular movements of the muscles of the legs and chest. With meticulous ether control such movements can be maintained for many seconds. During muscular activity cardiac and coronary reflexes also occur, as shown in the experimental records and data tables presented below.

Two examples are given in detail to illustrate this type of associated functions. In animal I, test 10, the first skeletal muscular contractions were moderately vigorous, somewhat irregular, and were allowed to continue for 65 seconds. The contractions were initiated without any visible external stimulus. They slowly decreased and ceased upon deeper etherization. The preliminary blood pressure was 154 mm. Hg and the heart rate 120 per minute. The new and unique feature of the test was the simultaneous development in the autonomic system of a pronounced reflex affecting the coronary system.

Protocol from dog 1, experiment 10.   Weight 6.8 kilos; right vagus cut at the jugular foramen and degenerated for 12 days, all other nerves intact; chest opened and artificial respiration; intravenous heparin 30 mgm. per kilo body weight.

During controlled light etherization skeletal muscular movements of the legs and chest occurred.   This reaction was associated with a pronounced coronary dilatation.   There was an immediate slowing of the heart rate which was accelerated slightly after the contractions of the skeletal muscles ceased.   There was some irregularity of blood pressure.   The respiratory rhythm was increased and the movements augmented.   The coronary dilatation began 23 seconds after the first muscular movement.   The dilatation was abrupt and prolonged,—lasting 300 seconds.

It is instructive that the heart rate during the period of muscular movement was slower than the preceding normal by 15 per cent during the first 5 seconds, and still by 3 per cent at 60 seconds.   Under deeper etherization the rate accelerated to 15,

TABLE 1

Data table from experiment 10, dog 1

TIME	BLOOD PRESSURE	PER CENT OF CHANGE	HEART RATE PER MINUTE	PER CENT OF CHANGE	FLOW FROM CORONARY SINUS	PER CENT OF CHANGE
	mm. Hg					
Normal	154		120		53	
15 seconds later	142	−8	102	−15	53*	Still normal
40 seconds later	173†	12	108	−10	80‡	51
60 seconds later	166	8	114	−3	91	72
90 seconds later	140	−9	138	15	69	30
140 seconds later	144	−7	144	20	64	21
175 seconds later	143	−7	156	30	62	17
240 seconds later	144	−7	144	20	56	5
300 seconds later	144	−8	144	20	50	−6
340 seconds later	144	−8	144	20	56	5

* Coronary flow still normal at 15 seconds.
† Blood pressure irregular at this time.
‡ Coronary dilatation began 23 seconds after first muscular movements.

20 and 30 per cent, measured at 90, 140 and 175 seconds.   The last two minutes the rate was constant at 20 per cent over the initial heart rate.

The coronary response was a primary dilatation that began about 23 seconds after the first muscular movements.   The dilatation rapidly mounted to a 72 per cent increase at 60 seconds, measured by the increase of flow of blood from the coronary sinus.   The dilatation slowly disappeared until the initial normal flow was reached in 300 seconds.   The form of the curve of rapid development and of slow decline of the dilatation is very typical of smooth muscle response.   The rate of coronary flow was independent of, i.e., did not follow, the curve of variation of either blood pressure or heart rate.   One concludes that the coronary dilatation is an independent function carried out by its own reflex nerve controls, though initiated in close association with the voluntary muscular movements.   The coronary dilatation began while the heart rate was 10 per cent slower than normal.   The volume of the initial coronary dilatation was rapidly increased and out of all proportion to the fluctuations of blood pressure.   In fact the blood pressure is relatively constant and lower than the preactivity normal except at the 40 and 60 second periods.
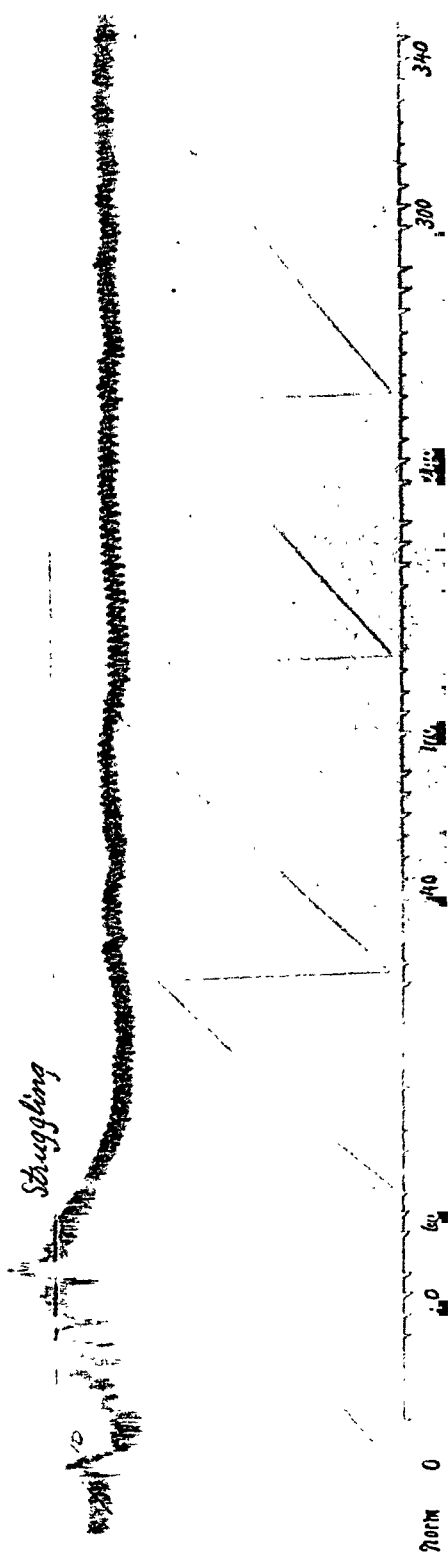
Fig. 1. Coronary dilatation associated with muscular movements under light, or sub-surgical, etherization. The top line records signals, the second line blood pressure by mercury manometer, the third measures the rate of blood flow from the coronary sinus by the author's volume manometer, time in 5 seconds and minute intervals. The numerals indicate time in seconds from the initial movement. The figure is reduced to 42 per cent of the original.
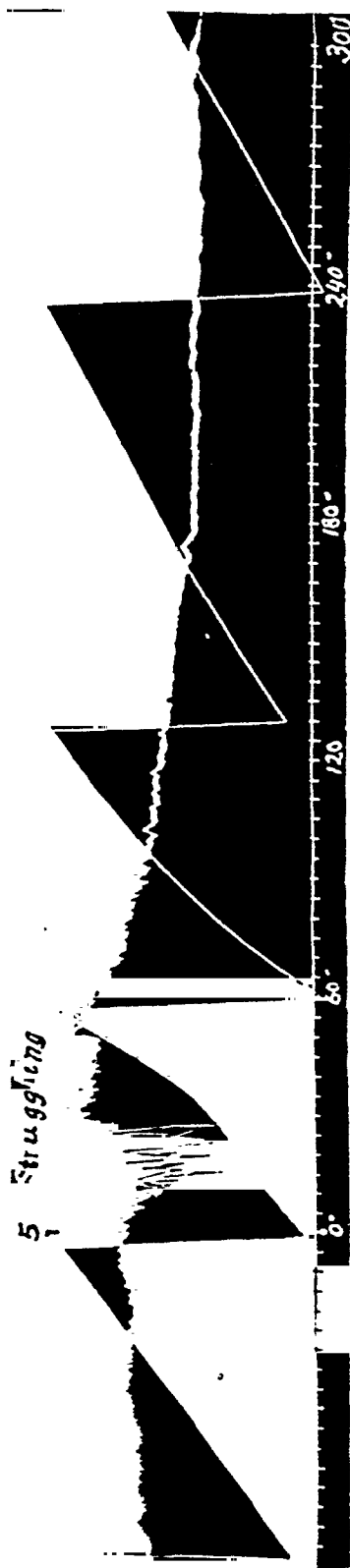
Fig. 2. A second example of response of the coronary blood vessels to reflexes associated with contractions of skeletal muscles. The legends of figure 1 apply also to this figure.

The coronary and the cardiac innervation paths of this animal are all intact except for the right vagus nerve. The cardiac slowing obviously takes place by reflexes through the left vagus. No coronary constrictions occur, hence the coronary control is not modified by the degeneration of the right vagus. All efferent dilator nerves are intact hence the dilatation is the terminial event of nerve reflexes reaching the coronary vessels by way of the thoracic and cervical pathways. This experiment demonstrates that the coronary reflexes associated with developing activity of skeletal muscles are primarily dilator and not constrictor in type. This proof is of great significance and should be emphasized.

TABLE 2

Data table experiment 5, dog 2

TIME	BLOOD PRESSURE	PER CENT OF CHANGE	HEART RATE PER MINUTE	PER CENT OF CHANGE	CORONARY FLOW; CORONARY SINUS	PER CENT OF CHANGE
	mm. Hg					
Normal	105.5		156		50	
5 seconds after	106.3	−1	144	−8	52.5	5
15 seconds after	85	−19	54	−65	49	−2
30 seconds after	91	−14	102	−35	45.5	−9
40 seconds after	124.5	18	150	−4	85	70
50 seconds after	121	15	144	−8	100	100
55 seconds after	131	24	150	−4	105	110
65 seconds after	115.5	9	156	0	100	100
75 seconds after	105	0	162	4	79.5	59
90 seconds after	97.3	−8	171	9	65	28
105 seconds after	89.5	−15	180	15	53	6
120 seconds after	84	−20	174	11	42.5	−8
135 seconds after	79.5	−25	174	11	42.5	−15
150 seconds after	77	−27	174	11	40.5	−20
165 seconds after	72	−32	174	11	37.5	−25
180 seconds after	66	−38	171	9	34	−31
195 seconds after	64	−39	168	8	33.5	−33
210 seconds after	63.2	−40	168	8	33.5	−33
240 seconds after	64	−39	168	8	36	−28
270 seconds after	63	−40	171	9	36	−28
300 seconds after	64	−39	162	4	37	−26
360 seconds after	63	−40	168	8	34.4	−31

A second experiment of like import runs more or less parallel with that given above, but a greater disturbance occurs. In this second animal the entire nervous system was intact. The observation occurred early in a long series of other tests, when the physiological mechanisms are more sensitive than later. The records and tabulated data are offered herewith.

Protocol of dog II, test 5. Weight 5.5 kilos, all nerves intact; ether; heparin; open chest, artificial respiration.

Under light etherization independent movements of skeletal muscles developed lasting 55 seconds. The data from this test are given fully in table 2 above.

Blood pressure was very irregular from the onset of the test and during muscular contractions. It decreased during the early period of 30 seconds then suddenly increased 18 per cent above the initial normal, at the 40 second period. After the contractions were controlled by ether the pressure was not sustained at a constant level, as in test 1, but continued to fall slowly for 5 minutes before reaching a constant level.

There was profound slowing and irregularity, i.e., reflex inhibitory slowing, of the heart rhythm through the first 40 seconds, then a return to the preceding normal followed by a mild acceleration as the etherization was increased.

The coronary flow was passively lowered a bit during the greatest decrease of blood pressure but at the 35 second period a sharp and active dilator reflex began. The coronary dilatation reached a maximum of 110 per cent at 55 seconds. It returned to the normal between 105 and 120 seconds. In the after period the flow gradually decreased to its lowest level at minus 31 per cent after 360 seconds, then it began again to dilate. The acute dilatation in this test was also primary, as in animal I. The coronary response bears all the characteristics of a reflex autonomic dilatation. It has the significance of a compensating reflex. The blood pressure passively influences the rate of coronary flow but the two phenomena are obviously not parallel functions in this experiment.

The coronary reflex dilatations in association with contractions of voluntary muscle are significant from a somewhat different point of view, viz., they are primary dilator reflexes. This fact supports earlier observations of the writer, that coronary reflexes in normal animals are primarily dilator in type (1, 2). They demonstrate that the acute coronary dilator reflexes are developed by the conditions that stimulate skeletal muscle movements.

The tests described in this paper furnish acute experiments which give the clue for the final interpretation of the facts developed by the ingenious method of the electric stromuhr measurements of coronary flow in the dog published by Essex, Herrick, Baldes and Mann (3). These authors say in discussing the profound coronary dilatations they obtained (p. 623), "The evidence indicates other influences as important as blood pressure in augmenting coronary blood flow when a dog is given successive increments in the rate of work".

It is well established that variations in blood pressure passively affect the rate of coronary flow. But for the experiments quoted above giving the effects of treadmill work on coronary flow may we offer the explanation that associated reflex coronary dilatations are in fact the "other influences" actively augmenting the coronary flow beyond the influence of the passive factor of variations in blood pressure.

SUMMARY

The observations presented in this paper and their physiological significance are condensed into the following:

1. Under light etherization skeletal muscular movements may be induced and controlled for brief periods.

2. During skeletal muscular activity blood pressure is more or less variable, with a tendency to rise in the later stage of the skeletal muscular activity and to fall in the after-period.

3. The heart rate shows a variable degree of reflex slowing at the very beginning of the movements, but quickly recovers and is accelerated in the after-period.

4. The coronary flow is sharply augmented in a primary reaction. The dilatation persists into the after-period. The coronary dilatation is an associated reflex of the greatest significance to cardiac nutrition during the added heart strain incident to skeletal muscular activity.

5. The coronary reactions were determined by the Morowitz technic and continuous measurement by the author's flow manometer.

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THE PRESENCE AND DISTRIBUTION OF HISTAMINE IN BLOOD[1]

DAVID MINARD

From the Departments of Physiology of The University of Chicago and of the University of Illinois College of Medicine

The development of new procedures of extraction has established the presence of histamine in normal blood from a number of mammalian species (1) (2). Code (3) in studying the distribution of histamine in blood concludes that the activity is associated largely (80–90 per cent) with the white cells, particularly the granulocytic elements. Although confirming Code's observations on the relatively high histamine content of the white cell layer, Anrep et al. (4) ascribe only about 50 to 60 per cent of the total histamine content of rabbit blood to this fraction, the remainder being associated with the red cells and plasma. A preliminary study (5) led us to the conclusion that, within the limits of accuracy of the methods employed, 97 to 98 per cent of the histamine content of rabbit blood is in the blood platelets. More recently an independent investigation by Zon et al. (6), utilizing the unique method of rendering rabbits platelet-free with antiplatelet serum, has led to essentially the same conclusion.

In the present communication I propose to describe a method for the extraction of blood histamine which is more rapid and less tedious than methods used at present and to elaborate on the observations previously reported.

EXPERIMENTAL PROCEDURE. Rabbits and dogs served as donors of the blood used in these experiments.

Collection of blood samples. Rabbit: Samples were obtained from the lateral vein of the ear, which had previously been shaved, made hyperemic by heating with an electric bulb, and coated with vaseline. The vein having been nicked with a razor, the blood was allowed to drop directly into the anticoagulant contained in a 15 cc. graduated centrifuge tube, extreme precautions being taken to prevent coagulation. In the majority of earlier experiments 0.07 cc. of 5 per cent sodium citrate per cubic centimeter of blood was used and made up to a volume equal to that of the blood to be obtained with calcium-free Ringer's solution. In later experi-

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[1] The major portion of this paper constituted part of a dissertation for the degree of Doctor of Philosophy in Physiology at the University of Chicago.

ments, when platelet volumes were to be measured, 1.3 per cent sodium oxalate served as the anticoagulant. In a few experiments on undiluted blood heparin was employed. The tube containing the anticoagulant was chilled in an ice bath and immediately replaced after collection of the sample.

All samples of plasma used were prepared by centrifuging the blood for 20 minutes in a refrigerator room. In later experiments to avoid contamination of the plasma samples with platelets the plasma was removed from the precipitated cells and recentrifuged another 20 minutes.

Platelet-rich, cell-poor suspensions were obtained by centrifuging 10 cc. of the diluted blood (1:1) for one and one-half to two minutes, at 2,300 r.p.m. Quantitative platelet volume studies by the thrombocytocrit method of Van Allen (7) showed such suspensions to contain from 50 to 85 per cent of the platelet volume of the original diluted blood, while over 99 per cent of the red cells and 95 to 100 per cent of the white cells were absent. The platelet concentration in the centrifuged specimens was determined by diluting that quantity of suspension obtained from 1 cc. of the original blood to 5 cc. with the oxalate solution, thus making possible direct comparison of the platelet volumes in the suspension and in the original blood which is diluted 1:5 with the oxalate solution in the Van Allen procedure.

Dog: In the studies on dog blood samples were obtained by venepuncture, the syringe containing 0.5 cc. of 5 per cent sodium citrate for 10 cc. of blood. Platelet suspensions were obtained as above except that larger quantities of blood were used. Thirty cubic centimeters of blood were drawn into a syringe containing 30 cc. of 1.3 per cent sodium oxalate, mixed, and centrifuged three minutes. The pink, cloudy plasma was then removed and recentrifuged for three minutes to throw down most of the remaining corpuscular elements. After a 2 cc. sample of this suspension had been taken for a thrombocytocrit determination, 25 cc. of that remaining was centrifuged for one hour, the clear plasma decanted off, and the precipitated mass of platelets stirred up in 5 cc. of calcium-free Ringer and extracted in parallel with the whole citrate blood sample.

Hematocrit determinations were obtained on all samples used.

Methods of extraction. Electrodialysis: MacGregor and Thorpe (8) describe an electrodialytic method for the rapid extraction of histamine from tissues. Although these authors failed to detect the presence of histamine in (human) blood, the high histamine content of rabbit blood suggested the applicability of this method to blood histamine studies in this species.

Four three-chambered cells were used similar in construction to those described by MacGregor and Thorpe, except that the middle chamber was made of pure gum rubber, thus eliminating the necessity of the rubber

gaskets. Parchment paper membranes separated the middle and electrodal chambers.

Twenty-five cubic centimeters of distilled water were used in each of the electrodal chambers and 20 cc. in the middle chamber. The blood samples to be dialyzed were placed in the middle chambers of the cells, care being taken that the same equivalent quantity of electrolyte was added to each cell. The smallest samples used were 0.5 cc. blood in 2.0 cc. of isotonic electrolyte and the largest samples 5 cc. of heparinized whole blood.

The four cells were connected in parallel to a 110 v. DC source, part of the current being shunted through electric light bulbs during the initial period of dialysis. Removal of the histamine was complete within about one-half hour, although the dialysis was continued in most cases for one hour as a precautionary measure.

After completion of the dialysis the cathodal fluids were removed, chambers washed three times, the combined fluid and washings neutralized with 0.2 NHCl against phenolphthalein, and each made to 50 cc. with distilled water. The dialysates were then ready for assay or further treatment.

Trichloracetic acid extraction: As a check several experiments on rabbit's blood were conducted using the procedure described by Barsoum and Gaddum, samples being collected by allowing the blood to drop directly into weighed tubes containing the 10 per cent trichloracetic acid. Samples for dialysis were obtained at the same time.

In the dog experiments, trichloracetic acid extraction was used exclusively, since the low histamine concentration in dog blood required a quantity in excess of that which the electrodialytic cells could adequately handle.

Methods of assay. Isolated guinea-pig intestine: Routine histamine assays of the blood dialysates were made using the isolated guinea-pig intestine as the test object.

The tissue bath and heater used were essentially the same as described by Burn (9) with the exception that the Ringer-Locke solution was led through a glass coil inside the heating chamber before entering the bath, whose capacity was 25 cc. in most experiments.

It was found that the intestine obtained from a guinea pig starved 24 hours could be preserved in Ringer-Locke solution at ice box temperatures for as long as seven days with little diminution in sensitivity to histamine. After a recovery period in an aerated bath (37°C.), during which constant histamine doses produced gradually increasing contraction heights, strips refrigerated from two to seven days yielded considerably more regular and uniform responses than fresher specimens. Spontaneous activity of the isolated strip, a factor of some annoyance in this method of assay, was almost wholly absent in the preserved material. It might also be

added that the use of preserved intestinal strips renders the frequent sacrifice of costly guinea pigs unnecessary.

The standard used was a solution of histamine di-phosphate equivalent to a 0.1×10^{-6} solution of the basic amine. Only after successive checks with varying doses was the assay of any sample said to be complete. All assays have been within the limits of accuracy of ± 5 per cent, while in some experiments we feel justified in reporting an accuracy of ± 2.5 per cent.

Blood pressure of the etherized cat: In a few experiments effects of samples were compared with those of a histamine standard (1.0×10^{-6}) on the blood pressure of the etherized cat before as well as after administration of atropine.

RESULTS. *Rabbit.* Whole blood: Quantitative estimations of histamine equivalents in electrodialysates of blood from several rabbits have given a range of values varying from 1.8×10^{-6} to 5.0×10^{-6} of the basic amine for the original blood. Samples from the same rabbit taken at intervals of several days showed less variation.

1. In a series of control studies 12 samples were dialyzed in duplicate, six samples in triplicate, and four samples in quadruplicate. In all cases but one agreement was within the limits of accuracy of the assay. Redialysis of the middle chamber liquid for one hour and concentration of the resulting cathodal liquid revealed complete absence of the active substance.

2. More than 90 per cent of 5 gamma of histamine added either alone to the middle chamber or to the blood sample to be dialyzed could be recovered (six experiments).

3. The active substance was stable to prolonged boiling in NHCl.

4. Close quantitative agreement was observed between electrodialysates and trichloracetic acid extracts of duplicate samples.

5. Atropine added to the tissue bath in no way affected the final result of the assay. Hence atropine was not routinely used in these experiments.

6. Incubation of the electrodialysates with an equal volume of a 1:20 extract of dried kidney powder in phosphate buffer for 1.5 hours at 37°C. resulted in complete loss of activity. Kidney extract, as pointed out by Best and McHenry (10), is a rich source of histaminase.

7. Recently thymoxyethyl diethyl amine has been shown to abolish specifically the action of histamine on the isolated guinea-pig intestine (11). This drug was found to reduce the action of histamine and the blood dialysate on the isolated intestine to the same extent, whereas the effects of KCl and acetylcholine were altered but slightly.

8. Neither the kind of anticoagulant used (sodium citrate and sodium oxalate), nor the amount of electrolyte employed in diluting the blood seemed to have any effect on the final results. Isotonic sodium oxalate, contrary to Code's findings with solid potassium oxalate as an anticoagu-

lant (3), appeared to alter neither the total extractable histamine nor its distribution in the blood.

9. In agreement with Barsoum and Gaddum's findings (1) no significant change in the histamine content of the blood occurs on standing. In seven experiments a sample of blood dialyzed immediately was compared with a duplicate sample dialyzed after standing four hours at room temperature. In two experiments the older sample showed about 5 per cent decrease in activity. No changes occurred in samples kept for fourteen hours at ice-box temperature.

Plasma, platelets and cells: In preliminary studies, inconsistent results were obtained in the determination of the relative histamine contents of plasma and formed elements, dialysates of plasma frequently giving much higher histamine values than indicated by direct application of the plasma to the strip. Further, plasma from samples briefly centrifuged contained more histamine than plasma obtained after prolonged centrifuging. Microscopic examination of the briefly centrifuged plasma samples revealed platelets in large numbers, although the cellular elements were almost entirely absent. This fact led to the following findings:

1. In two experiments electrodialysates of a mass of platelets obtained by prolonged centrifuging of a platelet-rich, cell-free suspension possessed 65 to 75 per cent of the whole blood histamine content. The precipitated cellular elements, though washed repeatedly, showed 15 to 20 per cent of the total content with 5 per cent in the plasma. Although these experiments indicate a large portion of the histamine to be in the platelets, it was obviously impossible either to recover all the platelets by this method or to wash the cells free of platelets.

2. Using the thrombocytocrit technique of measuring platelet volumes, we could demonstrate that within narrow limits the ratio of the histamine contents of a platelet suspension and of whole blood is the same as the ratio of their platelet volumes. In five experiments the two ratios agreed to well within 10 per cent. In one experiment the difference was 18 per cent (see table 1).

3. Eight thrombocytocrit determinations in five different rabbits gave an average value of 0.85 per cent (range 0.70–1.27 per cent).

4. Application was made of the principle employed in the thrombocytocrit method of Van Allen, namely, that when blood is diluted five times with an isotonic electrolyte solution (1.3 per cent sodium oxalate) and allowed to stand, the cellular elements settle out leaving the platelets in a uniform suspension for several hours in the same concentration as existed in the blood before sedimentation. In nine experiments close quantitative agreement was found between the histamine concentrations of dialysates of unsedimented blood, the cell-free platelet suspension, and the sedimented layer of cells, in all of which the platelet concentration is

the same although the cellular contents vary widely. As stated above, dilution and standing alter neither the total extractable histamine nor the plasma histamine level (see record).

TABLE 1

Comparison between platelet volumes and histamine concentrations of whole blood and a cell-free platelet suspension

EXPERI-MENT NUMBER	THROMBOCYTOCRIT (CC. PLATELETS/100 CC. WHOLE BLOOD)			HISTAMINE CONC., γ/CC.			PER CENT DIFFERENCE
	A Whole blood	B Platelet suspension	A/B	C Whole blood	D Platelet suspension	C/D	
1	0.70	0.48	1.45	5.5	3.8	1.44	1
2*	0.88	0.64	1.37	3.0	2.25	1.36	1
3	0.94	0.77	1.22	1.8	1.2	1.50	18
4	0.76	0.66	1.15	3.0	2.5	1.20	4
5	1.27	0.98	1.29	3.7	3.0	1.23	5
6	0.90	0.77	1.16	2.5	2.1	1.19	2.5

* For assay, see record.

Fig. 1. Isolated guinea-pig intestinal strip, preserved four days in Ringer's solution at ice box temperature; capacity of bath 25 cc. A = whole blood, dialyzed immediately; B = whole blood, dialyzed after 3½ hours standing at room temperature; C = cell-free platelet suspension, prepared by sedimentation of cells (throm'crit = 0.88 per cent); D = cell-free platelet suspension, prepared by differential centrifugation (throm'crit = 0.64 per cent); E = recentrifuged plasma; Hi = histamine, 0.1×10^{-6} of basic amine.

Note: Each cubic centimeter of electrodialysates A, B, C and D represents 0.02 cc. of original blood or platelets therefrom; each cubic centimeter of electrodialysate E represents 0.04 cc. of plasma.

5. In fifteen experiments the average histamine concentration in plasma was 2.8 per cent of that in whole blood. In most instances the plasma dialysates were concentrated ten times before assay.

6. Electrodialysates of platelet suspensions, plasma, and whole blood, as well as trichloracetic acid extracts of whole blood, when concentrated and assayed against histamine on the blood pressure of the cat gave values which checked quantitatively to about 10 per cent of those obtained with the guinea-pig intestinal strip. The values were the same before and after administration of atropine to the cat.

Dog. Using the trichloracetic acid extraction procedure and the guinea-pig intestinal strip method for assay, we have found that in the dog, contrary to our findings in the rabbit, the platelets contain a relatively small proportion of the total blood histamine. In these experiments the values for the equivalent concentration of histamine in whole blood ranged from 0.035×10^{-6} to 0.025×10^{-6}. The corresponding histamine values for the platelets ranged from 0.7γ to 0.5γ per cc. of platelets. Since the platelet volume is about 1 per cent of the whole blood, the platelets would account only for about 15 per cent of the total histamine equivalent of the blood.

DISCUSSION. The quantitative extraction of blood histamine in the rabbit can readily be achieved by electrodialysis in a manner more rapid and less tedious than the trichloracetic acid procedures in current use. The active substance in the blood dialysates resembles histamine in its physiochemical as well as in its pharmacological properties. Since histamine has been isolated chemically from the blood of the rabbit (12), it may be safely assumed that the active material is histamine itself.

Application of this method to studies on the distribution of histamine in rabbit blood has led to the finding that the histamine present is localized to a large extent (97–98 per cent) in the platelets. Aside from a small portion (2–3 per cent) in the plasma, which might conceivably result from unavoidable breakdown of the histamine-containing platelets, the remaining constituents of blood contribute so little to the total histamine content as to be undetectable by the methods employed.

As already indicated, these findings are at variance with those of Anrep et al. The discrepancy may possibly find explanation in the failure of these authors to prevent partial agglutination and breakdown of the platelets. Code (3) ascribes the major portion of the histamine in rabbit blood to the white cell layer of centrifuged blood (which contains most of the platelets), but suggests that the leucocytes are the histamine-containing elements.

No support has been found for the distinction raised by Tarras-Wahlberg (13) between a free-phase and a bound-phase of the histamine in rabbit plasma. From the high values he cites for the histamine content of

plasma, it seems likely that his samples were not completely free of platelets, a view which is borne out by the method he describes for obtaining plasma. Zon and his collaborators have conducted *in vivo* experiments in the rabbit which leave little doubt that the platelets are the chief histamine source in this species.

Although the present studies are limited largely to the rabbit, a few experiments on dog blood have made possible the conclusion that in this species, contrary to the findings in the rabbit, the platelets contribute relatively little to the total blood histamine. Hence it would seem that there exists a qualitative as well as a quantitative difference in the histamine content and distribution in the blood of the species studied. Therefore, one must avoid applying too broadly conclusions based on blood histamine studies in single species.

On the basis of platelet volume and platelet histamine determinations, a fact of interest appears, namely, that rabbit platelets contain approximately 400 gamma of histamine per cubic centimeter. This is a value far in excess of the figures cited by various authors for other tissues. The possibility exists that the high histamine concentration in lung tissue, on the one hand, and in the platelets on the other, may be more than coincidental, since Howell and Donahue (14) have recently shown that the lungs are the principal source of these blood elements.

SUMMARY AND CONCLUSIONS

1. A modified method for the extraction of blood histamine by electrodialysis and assay on preserved isolated guinea-pig intestinal strips is described.

2. Quantitative studies have been made on the presence and distribution of histamine in blood of the rabbit and dog. Evidence is presented which supports the identity of histamine with the active substance in electrodialysates of rabbit blood.

3. The histamine activity of rabbit blood is located almost exclusively in the platelets. In dog blood, on the other hand, the platelets possess only a small share of the total blood histamine.

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BIOCHEMICAL CHARACTERISTICS OF DENERVATED SKELETAL MUSCLE, AT REST AND AFTER DIRECT STIMULATION

R. LEVINE, O. HECHTER AND SAMUEL SOSKIN

From the Department of Metabolism and Endocrinology,[1]
Michael Reese Hospital, Chicago

The atrophy of muscle which occurs following injury to the anterior horn cells in poliomyelitis and which can be duplicated experimentally by section of the motor nerve connections, consists in the final analysis of a loss of muscle protein and its replacement by fibrous tissue and fat. From a dynamic standpoint this must result from either an increased proteolysis, a decreased protein synthesis or both. The disturbance in protein metabolism may be primary or secondary.

The present state of knowledge does not permit of a decision as to whether the muscle atrophy following denervation is due to a primary failure in protein metabolism, or whether the latter is secondary to a disturbance in the carbohydrate cycle. The second alternative offers the most feasible approach to the problem in view of the relatively advanced state of knowledge of the subject and the existence of more adequate methods of investigation. The relevant literature (up to 1939) has been collected and reviewed by Tower (1). Her survey shows that different phases of the biochemistry of denervated muscles have been studied by a number of workers in various ways. However, no study has appeared in which the many observations have been made on the same species of animal under the same conditions; nor has it been attempted to link the observed biochemical changes in a causative manner with the muscular atrophy which follows denervation. We wish to report such an attempt.

I. THE LEVELS OF CARBOHYDRATE AND PHOSPHATE COMPOUNDS IN NORMAL AND DENERVATED MUSCLE. *Methods.* The animals used were healthy adult rats of both sexes, whose weights ranged from 180 to 220 grams. The diet consisted of "Purina" Fox Chow supplemented with 10 per cent powdered skim-milk. Two animals were kept per cage during the experimental periods.

The muscles of the hind limbs were denervated by sectioning the sciatic and femoral nerves on one or both sides depending upon the type of experi-

ment. The normal limb served as a control for the unilaterally denervated animals. Normal animals of the same weight and sex were used as controls for the bilaterally denervated rats. In all the experiments the gastrocnemius muscles were used for chemical analysis, at various intervals after the motor innervation had been severed. These intervals will be indicated in the text.

The various chemical determinations were done by the following methods:

1. *Inorganic and creatine phosphate:* Fiske and Subbarow (2) as adapted to the photoelectric colorimeter.

2. *Adenosine triphosphate:* Hydrolysis in N HCl at 100°C. for 7 minutes (Lohmann, 3).

3. *Hexosemonophosphate:* Formation of the water soluble barium salt (Needham, 4).

TABLE 1

SUBSTANCE	18 NORMAL RATS			20 BILATERALLY DENERVATED 14 DAYS			PER CENT DIFFERENCE
	Min.	Max.	Ave.	Min.	Max.	Ave.	
Glycogen.....................	400	700	507	100	350	241	−52.5
Lactic acid....................	14	33	24	16	44	27	
Creatine phosphate............	50	56	53	30	46	38	−28.3
Inorganic phosphate...........	22	34	26	17	31	21	
Adenosine triphosphate........	31	41	37	18	33	25	−33.0
Hexosemonophosphate..........	9	18	14	10	19	16	
Hexosediphosphate.............	5	12	8	6	17	10	
Total acid soluble phosphate....	152	207	194	131	190	176	

All values are in milligrams per 100 grams of muscle.

4. *Hexosediphosphate:* Hydrolysis in N HCl at 100°C. for 100 minutes (Lohmann, 3).

5. *Total acid soluble P:* Ashing with H_2SO_4 and H_2O_2 (Needham, 4).

6. *Glycogen:* Good, Kramer and Somogyi (5).

7. *Lactic acid:* Adaptation of micromethod of Miller and Muntz (6).

Results. The data are summarized in table 1. These results show a significant change in three substances. The glycogen concentration in the denervated muscle is less than half of the normal value. The creatine phosphate and adenosine triphosphate contents of denervated muscle are decreased by one-third as compared with normal control muscles.

Comment. Our results are in agreement with those of Hines and Knowlton, (7) (8) who found normal values for total acid soluble phosphate, low values for creatine phosphate and adenosine triphosphate and small changes in the inorganic phosphate content. Zanghi (9) also showed the

fall in creatine phosphate of denervated muscle and observed that it occurred after fibrillation has set in. There is agreement also as to the fall in glycogen content of paralyzed muscle (7).

This picture of the altered resting values of glycogen, creatine phosphate and adenosine triphosphate does not by itself throw any light on the dynamics of the glycolytic system. Are these quantitative changes associated with significant alterations in the enzyme systems operating in the glycolytic scheme? To answer this question we have investigated the ability of denervated muscle to phosphorylate carbohydrates and produce lactic acid from them.

II. PHOSPHORYLATION AND GLYCOLYSIS OF NORMAL AND DENERVATED MUSCLE IN VITRO. *Methods.* Muscle extracts were prepared according to the method of Kalckar (10) with M/30 Sorensen phosphate buffer. These extracts were incubated with the desired carbohydrate at 37.5°C.

TABLE 2

SUBSTRATE	NO. OF EXPERI- MENTS	MGM. P ESTERIFIED IN 60 MINUTES					
		Normal limb			Denervated limb		
		Min.	Max.	Ave.	Min.	Max.	Ave.
Glycogen........	6	0.33	0.60	0.51	0.18	0.39	0.30
Glucose....................	3	0	0	0	0	0	0
Fructose....................	3	0	0	0	0	0	0
Glycogen + glucose........	4	0.24	0.36	0.30	0.17	0.22	0.19

for varying periods of time. NaF $\left(\dfrac{M}{200}\right)$ was used to inhibit dephosphorylation.

The in vitro production of lactic acid by the muscles was investigated by preparing a muscle brei and incubating it with glycogen under anaerobic conditions. A typical vessel contained: 1, 200 mgm. of muscle brei; 2, 25 mgm. of glycogen; 3, 2.5 cc. Hastings-Ringer solution buffered with M/15 phosphate buffer; 4, the gas phase was N_2.

Results. Table 2 shows that denervated muscle (14 days after nerve section) exhibits a lessened ability to phosphorylate glycogen. Neither the normal controls nor the paralyzed muscles are able to phosphorylate glucose or fructose. But both of these hexoses inhibit the phosphorylation of glycogen by muscle (table 2). The rate of anaerobic lactic acid production (table 3) lies in the same range in both normal and denervated muscles. The same holds for the rate of disappearance of glycogen (table 3).

Comment. It is apparent that the mechanism for the production of lactic acid from glycogen is not impaired despite the apparent lower

phosphorylosis of glycogen. Since this process involves all the enzyme systems known to be involved in glycolysis, no serious deficiency in any of these systems can exist.

The question then arises as to whether the difference between denervated and normal muscle in regard to the levels of glycogen, creatine phosphate and adenosine triphosphate, may not be due to a disturbance in the oxidative systems present in the tissue. Therefore we have studied the overall O_2 consumption, the succino-oxidase activity and the presence of other common dehydrogenase systems.

III. IN VITRO RESPIRATION OF NORMAL AND DENERVATED MUSCLE. A. *Oxygen Consumption. Methods and results.* Respiration was measured in Barcroft-Warburg manometers. Temperature of the bath: 37.5°C. Gas phase: 100 per cent O_2. Medium: Hastings-Ringer's solution.

TABLE 3

NO. OF EXPERI-MENTS	PER 1 GRAM OF MUSCLE	NORMAL LIMB		DENERVATED LIMB	
		30 min.	60 min.	30 min.	60 min.
6	Mgm. lactic acid formed	4.15	6.25	4.00	5.98
6	Mgm. glycogen broken down	6.10	17.25	6.55	14.85

TABLE 4

NO. OF EXPERIMENTS	DETERMINA-TION	PREPARATION	NORMAL LIMB	DENERVATED LIMB
6	Q_{O_2}	"Strips"	1.8–2.7	1.5–2.4
10	Q_{O_2} (Succ.)	Brei Homogenized tissue	3.8–6.7 4.5–6.2	2.1–4.5 4.7–5.5

Our data in table 4 confirm the previous work by Knowlton and Hines (11) and E. Fischer (12) in showing that the in vitro O_2 consumption of denervated muscle differs little if at all from the normal. Because denervated muscle has a greater proportion of fibrous tissue than normal, and because oxygen consumption is expressed in terms of muscle weight, the above data suggest that the O_2 consumption of the active cells of the denervated muscle is actually increased.

Comment. The measurement of metabolic reactions in vitro does not give a quantitative picture of events in vivo. Although it is evident that the capacity of muscle tissue to use up O_2 is not impaired by denervation, this does not necessarily indicate that the O_2 is used normally. The total O_2 consumption of a tissue is the sum derived from all oxidative reactions occurring simultaneously. Despite normal values for Q_{O_2} the component reactions may be different. To obtain some information about this we

have determined the (1) succino oxidase activity and the (2) presence or absence of dehydrogenases for a number of metabolites.

B. *Succino-oxidase Activity. Methods and results.* The succino-oxidase activity was measured by the method of Elliott (13).

Table 4 shows that the succino-oxidase activity of denervated muscle exhibits a barely significant decrease as compared to normal muscle. Comment is reserved to the end of the next section.

C. *Dehydrogenases. Methods and results.* The presence of dehydrogenases for various substrates was tested by incubating muscle brei, suspended in Ringer's solution, in evacuated Thunberg-Keilin tubes. The main chamber contained 200 mgm. of muscle brei, 2 cc. of Ringer's solution and 0.2 cc. of $M/1000$ methylene blue; 0.2 cc. of a $M/100$ solution of the substrate to be tested was placed in the hollow stopper.

The metabolites examined in the above manner were as follows: glucose, lactic acid, pyruvate, fumarate, succinate, malate and citrate. There was no significant difference between the results obtained with denervated as compared to normal muscle.

Comment. Succino-oxidase activity provides some measure of the capacity of the cytochrome-cytochrome oxidase system. The activity of the dehydrogenases is involved in the first part of the oxidative chain.

Our data on succino-oxidase activity do not agree with those of Knowlton and Hines (11) who reported that the succino-oxidase activity is lowered about 50 per cent by denervation. However, their figures for the succino-oxidase were obtained with the methylene blue technique, so that it is hard to compare our results with theirs, as regards the normal or the denervated muscle. Our results indicate that denervation does not lead to either a quantitative or qualitative impairment of the oxidative capacity of the muscle fiber. The various dehydrogenase activities which we tested were present and the cytochrome-cytochrome oxidase system, though slightly decreased, was not impaired to a degree that would limit the oxygen consumption of the entire system. Thus the transfer of H_2 from substrate to molecular O_2 in paralyzed muscle proceeds through normal pathways despite the lack of nerve supply.

In view of the above it seemed logical to seek the explanation for the lowered levels of glycogen, creatine phosphate and adenosine triphosphate of denervated muscle, in terms of dynamic relationships which can be adequately studied only in vivo. For this purpose we undertook to examine the biochemical behavior of denervated muscle in the intact animal during work and recovery.

IV. THE METABOLISM OF NORMAL AND DENERVATED MUSCLE DURING WORK AND RECOVERY. *Methods.* For the investigation of the biochemical changes during contraction of the intact muscle in the living organism, we adapted the technique of Sacks (14). The gastrocnemius was dissected

free, under avertin anesthesia, without damage to its blood supply. It was then left at rest for 10 to 15 minutes, to allow for the restitution of any biochemical changes resulting from the trauma of the dissection. The muscle was then caused to work by stimulating it directly with a faradic current (induction coil) for 20 seconds. In a particular muscle the desired interval of recovery from the tetanic contraction was allowed (0, 10, 20, 30, 40 and 60 minutes). At the end of this period the muscle was quickly removed by severing its origin and insertion and immediately frozen between blocks of solid CO_2. The frozen material was weighed

TABLE 5

(72 animals; 408 determinations)

	DAYS AFTER DE-NERVA-TION	RESTING			IMMEDIATELY AFTER 20" TETANUS			RECOVERY PERIOD														
								10 minutes			20 minutes			30 minutes			40 minutes			60 minutes		
		Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
Glycogen	0	400	700	507	200	500	333	340	350	340				260	430	345				420	460	440
	1½-2	400	500	450	150	390	248	280	330	305	200	310	255				300	310	310	350	400	380
	4½	170	420	268	50	230	124	50	140	108	120	170	140							110	180	145
	14	100	350	241	70	330	150	190	190	190	160	160	160	200	200	200				170	170	170
Lactic acid	0	14	33	24	102	102	102	38	38	38	49	49	49	22	22	22	23	23	23	23	24	24
	1½-2	22	41	29	85	161	120	30	45	38	29	32	31				29	40	34	26	32	29
	4½	17	39	29	78	130	100	19	59	44	29	73	53							25	38	32
	14	16	44	27	43	88	63	46	46	46	30	30	30	31	31	31	18	19	19	32	33	32
Inorganic phosphate	0	22	34	26	32	43	41	20	31	26	22	23	23	19	23	21	21	21	21	23	24	24
	1½-2	17	31	23	29	58	43	26	26	26	24	30	27				22	27	24			
	4½	18	25	20	26	49	40	17	27	20	22	23	23							22	31	27
	14	17	31	21	23	51	30	25	25	25	27	27	27	19	19	19	25	27	26	22	23	23
Creatine phosphate	0	50	56	53	8	39	21	36	55	46	54	57	56	49	55	52	53	53	53	50	51	51
	1½-2	52	64	56	14	44	27	52	56	54	48	53	51				41	48	45	50	53	51
	4½	44	58	49	19	30	23	35	55	44	37	55	43							44	50	47
	14	30	46	38	15	31	22	30	30	30	28	28	28	31	31	31	34	35	35	34	34	34
Adenosine triphosphate	0	31	41	37	31	40	35	31	36	34	32	37	35	31	36	34	29	29	29	31	33	32
	1½-2	34	40	37	31	39	35	39	40	40	38	40	39				33	35	34	36	38	37
	4½	22	36	32	23	36	31	22	35	31	33	38	35							37	38	37
	14	18	33	25	21	27	26	26	26	26	21	21	21	28	28	28	33	33	33	32	32	32

and divided for various chemical determinations. The chemical determinations done included the following substances: glycogen, lactic acid, inorganic phosphate, creatine phosphate and adenosine triphosphate.

The comparison between the normal muscle and the paralyzed muscle, at the time when the latter might be expected to show the full effects of denervation, was made using normal rats and animals 14 days after bilateral denervation of the hind limbs. In order to observe the possible coincidence of chemical changes with the onset of fibrillation, which is known to start between 3 to 5 days after denervation, comparisons with the

normal were also made on rats which had been denervated for periods of $1\frac{1}{2}$ to 2 and $4\frac{1}{2}$ days respectively.

Results. The data are presented in table 5. The most significant changes are seen in connection with glycogen resynthesis. It is evident that glycogen breakdown and recovery is normal $1\frac{1}{2}$ to 2 days after denervation, before fibrillation has begun. By $4\frac{1}{2}$ days, which is shortly after the beginning of fibrillation, there is already a dramatic drop in the initial glycogen level and a pronounced impairment in the ability of the muscle to restore its glycogen. The longer interval which elapses between $4\frac{1}{2}$ and 14 days makes very little further difference in glycogen breakdown and resynthesis.

The other marked difference between the normal and denervated muscle is in the breakdown and resynthesis of creatine phosphate. Unlike the changes in the glycogen these effects do not appear sharply at the onset of fibrillation, but seem to follow and perhaps are consequent to those of glycogen. Fourteen days after denervation the resting level of creatine phosphate is definitely lower than normal and the rate of its resynthesis is slow.

The lactic acid and inorganic phosphate show only minor changes. This is to be expected as regards readily diffusible substances which rapidly equilibrate with the blood.

Adenosine triphosphate is definitely decreased after 14 days of denervation. It should be noted, however, that this substance does not change significantly during contraction and recovery even of normal muscle, and hence its rôle in our comparison of dynamics may be discounted.

DISCUSSION. Our results show that immediately after nerve section and for 3 to 4 days thereafter, the chemical constituents and reactions which we have investigated remain indistinguishable from the normal. This is true as regards the carbohydrate levels, the content of creatine phosphate, adenosine triphosphate, inorganic phosphate and lactic acid. Following stimulation of the muscle the changes in these compounds in the denervated muscle resemble those which occur in normal muscle both as regards direction and extent. This period corresponds to that during which no perceptible atrophy occurs and in which the muscle has been found to appear very nearly normal by gross and histological examination (1). But perhaps a more important known characteristic of this period is the fact that the muscle is truly quiescent for no filbrillation has yet begun. Thus in all respects it appears that the pathologic process which sooner or later follows motor nerve section is not the immediate result of the loss of the nervous connection but depends upon a secondary change which takes a number of days to appear.

The beginning of the period during which both anatomic and chemical changes appear seems to coincide with the onset of fibrillation (1, 15).

During this period the muscle content as regards glycogen, creatine phosphate and adenosine triphosphate decreases. The level of inorganic phosphate is normal or somewhat raised. The lactic acid content is normal. Following direct stimulation of the muscle the chemical changes which occur during tetanic contraction and recovery are qualitatively similar to those which occur in normal muscle, but quantitatively reduced.

Our further observations bearing on the possible reasons for the quantitative differences between normal and fibrillating denervated muscle do not reveal anything which could not be accounted for by the fibrillation. Thus the *in vitro* O_2 consumption per unit dry weight of denervated muscle (which has ceased fibrillating by the time measurements are made) compares to that of the normal. The succino-oxidase activity, which may be taken as a rough measure of the cytochrome-cytochrome oxidase system is somewhat reduced but would be adequate for even greater rates of oxygen consumption than those observed. Furthermore the various dehydrogenases which we tested by the Thunberg technique were found to be present and to yield activities comparable to those observed in normal muscle. There is therefore no reason to suppose that the oxidative mechanisms in the denervated muscle pursue pathways other than those established for normal muscle, and there is no evidence that the denervated muscle is different from the normal, once it has ceased fibrillating, except in so far as it may show the effects of previous prolonged work (low levels). In this connection it seems to us significant that many years ago Langley (16) observed that the *in vivo* O_2 consumption of actively fibrillating paralyzed cat muscle was greater than normal as judged by arterio-venous O_2 differences. In the light of our present work this indicates that in the intact organism the paralyzed muscle because of the continuous activity due to fibrillation, oxidizes its metabolites not only in a normal manner but at a rate greater than normal. An interesting analogy exists between such a muscle and the muscle exhibiting the increased metabolic activity consequent to hyperthyroidism. The hyperthyroid muscle has a low carbohydrate content and reduced levels of creatine phosphate (17, 18). It is also noteworthy that the same probable etiologic agent (fibrillatory muscle twitching) is also present, and that similar consequences, namely, loss of muscle substance and weakness, ensue.

On the basis of the above it is possible to construct the following working hypothesis. The severance of the motor innervation leads to a disorganized but increasing activity of the muscle fibres. This is followed by the depletion of the carbohydrate stores at a rate greater than can be restored by the blood transport mechanism and the synthetic processes in the muscle. The decreased amounts of intermediary metabolites resulting from a rate of catabolism which closely approaches the rate of supply, affords insufficient raw material for synthetic recovery processes. This

may very well apply to protein synthesis as well as to carbohydrate. In this connection it is known that the synthesis of the amino acids essential for complete protein structure necessitates the presence of keto acids, which result from carbohydrate breakdown and are necessary for the process of transamination (19).

It remains to be proven whether the chemical changes which accompany the fibrillation of the denervated muscle of the rat are indeed responsible for the muscular atrophy. While the present work was in progress Solandt and Magladery published an abstract (20) in which they reported that the administration of quinidine inhibited the fibrillation in the muscles of denervated rats without significantly influencing the rate of atrophy. Evaluation of their results must await the publication of their full data, but it is evident that the interpretation of their work may depend upon the manner in which quinidine abolishes fibrillation. We have done preliminary work on the influence of prostigmine and atropine respectively on the atrophy of denervated muscles. The actions of these materials are probably exerted on the physiological mechanism of fibrillation (acetylcholine (21, 22)). We have found that prostigmine, which increases fibrillation and atropine which does the reverse, respectively increase and decrease the rate of atrophy of the denervated gastrocnemius muscle of the rat.

SUMMARY AND CONCLUSIONS

1. The biochemical characteristics of denervated skeletal muscle, previously a subject of fragmentary reports, have been studied on the same species of animal (albino rat) and under the same experimental conditions. An attempt has been made to relate the observed biochemical changes to the muscular atrophy which follows denervation.

2. Fourteen days after denervation, at a time when significant atrophy has developed, the gastrocnemius muscle of the rat exhibits low levels of glycogen, creatine phosphate and adenosine triphosphate. There is no significant difference from the normal as regards the following: lactic acid, inorganic phosphate, hexose monophosphate, hexose diphosphate and total acid soluble phosphate.

3. After a similar period of denervation determinations of phosphorylation, glycolysis, in vitro O_2 consumption, succino-oxidase and dehydrogenase activities indicate that the transfer of H_2 from substrate to molecular O_2 in the denervated muscle proceeds through the normal pathways, and at a rate which is adequate for normal respiration.

4. An examination of the biochemical behavior of denervated muscle in the intact animal during work and recovery has revealed that glycogen breakdown and resynthesis is normal at $1\frac{1}{2}$ to 2 days after denervation, before fibrillation has begun. After the onset of fibrillation there is a

dramatic drop in the initial glycogen level and a pronounced impairment in the ability of the muscle to restore its glycogen after contraction. Similar but somewhat delayed effects have been observed as regards creatine phosphate.

5. Our observations do not reveal any biochemical changes in the denervated muscle which could not be accounted for by the onset and continuation of fibrillation. It is suggested that the atrophy of denervation is an "overwork" atrophy. Work in progress indicates that the atrophy can be increased or diminished by agents which cause an increase or decrease, respectively, in the degree of fibrillation.

We wish to thank Dr. Philip Lewin for his interest in and his encouragement of this work.

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THE DISTRIBUTION OF RADIOACTIVE ISOTOPES OF IODINE IN NORMAL RABBITS[1]

IRVING ARIEL,[2] WILLIAM F. BALE,[2] VINCENT DOWNING,[2] HAROLD C. HODGE,[3] WALTER MANN,[2] STANLEY VAN VOORHIS,[4] STAFFORD L. WARREN[2] AND HELEN J. WILSON[2, 5]

From the University of Rochester, School of Medicine and Dentistry, and the Strong Memorial Hospital

In this report we have surveyed the distribution of radioactive iodine in the tissues of the rabbit at various periods after intravenous injection in order that later experiments relating to the iodine metabolism may be more clearly defined.

The iodine content of various tissues of the rabbit has been studied using chemical methods (1). However, these procedures are not appropriate for studying the *rate of distribution* of iodine introduced into the rabbit (2). By the use of radioactive iodine the distribution rates may be obtained following the administration of small doses of iodine (3, 4). These values establish the normal ranges of distribution rates for a given tissue as well as the variations which normally occur in the distribution rates to different tissues. Clinical use of this method has already been of some value in certain types of human thyroid disease (3). However, once normal values for iodine distribution in a suitable experimental animal are determined, the techniques of experimental pathology can be applied to expedite the study of variations in iodine metabolism characteristic of disease.

Radioactive iodine is prepared by bombarding metallic tellurium or one of its compounds in the cyclotron with six and one-half million electron volt protons. The nuclear reactions may be written as follows:
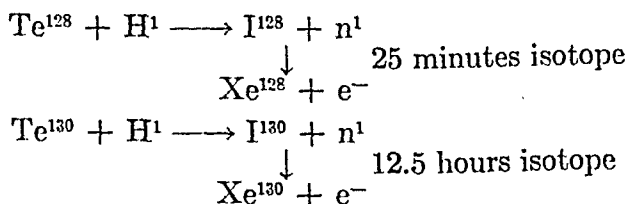
$$Te^{128} + H^1 \longrightarrow I^{128} + n^1$$
$$\downarrow \quad \text{25 minutes isotope}$$
$$Xe^{128} + e^-$$

$$Te^{130} + H^1 \longrightarrow I^{130} + n^1$$
$$\downarrow \quad \text{12.5 hours isotope}$$
$$Xe^{130} + e^-$$

Two radioactive isotopes of iodine with half-lives of 25 minutes and 12.5 hours, respectively, are thus formed. Isotopes with half-lives of longer duration are also produced but the isotope with a half-life of 12.5 hours predominates in the samples used.

Preparation of radioactive iodine for injection. Fifteen milliliters of concentrated C. P. nitric acid were placed in a 25 x 200 mm. test tube and the platinum foil bearing the tellurium and radioactive iodine dropped in along with about 5 mgm. or less of crystalline iodine. A stopper bearing a glass delivery tube was inserted and the delivery tube immersed in a narrow column containing carbon tetrachloride to absorb any iodine driven off. The nitric acid was heated nearly to boiling, poured into a small separatory funnel, and the iodine repeatedly extracted with ten milliliter portions of carbon tetrachloride until the acid layer was free from radioactive material as tested by placing the funnel near the ionization chamber of the counter. Another portion of the concentrated nitric acid was added to the foil and tellurium, brought to boiling temperature and the boiling continued until the tellurium dissolved. The subsequent extraction was repeated. The carbon tetrachloride fractions were united and extracted repeatedly with distilled water containing a few drops of a saturated sodium sulfite solution. The carbon tetrachloride fraction was discarded when it no longer contained any radioactive material. The excess sulfite was oxidized to sulfate with a few *drops of dilute solution of* potassium permanganate. The solution containing the radioactive iodine was evaporated to a volume of 25 ml., a 1 ml. aliquot removed and made up to 10 ml. for a radioactive standard from which the composite decay curve of the mixed iodine isotopes was determined.

Experimental procedure. Normal, young, adult stock, New Zealand, white rabbits were used throughout these experiments. These rabbits were all from the same stock, weighing 2.5 to 3.0 kgm., and were approximately of the same age (5-6 mos.). They were kept separately in wire-bottom cages and fed oats and dry alfalfa for 2 to 3 weeks before use, and maintained on this diet after the injection of radioactive iodine. The city water contains approximately 5 parts of iodine per billion, and no iodine was added to it.

Ten milliliters of the iodine-bearing solution were injected slowly into an ear vein of a rabbit. No untoward reactions occurred. At intervals the animals were sacrificed by injecting air into an ear vein, and samples of various tissues taken. The weight of each entire organ was determined and a weighed aliquot taken for examination. The sample was minced and dissolved in dilute NaOH with heat. The solution was then diluted to 10 ml., and a 2 ml. aliquot portion placed in the cup of the ionization chamber of the Geiger-Muller scale-of-four counter (5) for a determination of the radioactivity. The urine volume was determined and a 2 ml.

aliquot counted. For blood measurements, 2 ml. of serum were used in each instance. The counting procedure has been described in detail elsewhere (5).

In a series of experiments, aliquots of radioactive iodine having a known activity were dissolved in 10 per cent NaOH in some instances and in others placed in the NaOH solution in which approximately 1 gram of organic material had been ashed. The counts in both instances were identical, demonstrating that there is no appreciable absorption of beta rays by the ash of the organic material.

DATA. Table 1 gives a detailed statement of the various determinations. In the first column at the left are listed the individual tissues stud-

TABLE 1

Distribution of radioactive iodine in rabbit tissues at various times after injection (per cent dose per gram wet tissue)

	5 MINUTES	5 MINUTES	35 MINUTES	1 HOUR	1 HOUR	2 HOURS	2 HOURS	4 HOURS	8 HOURS	9 HOURS	12 HOURS	19 HOURS	24 HOURS	48 HOURS
Thyroid	1.2		4.1	8.2	0.75	16	23	0.97		1.43	2.4	3.2	2.4	15
Blood	0.16	0.12	0.10	0.01	0.09	0.08	0.06	0.11	0.11	0.05	0.08	0.003	0.02	0.06
Urine	0.19		0.26	0.03		0.04	0.06	0.02	0.05	0.31	0.12	0.11	0.08	0.06
Liver	0.01		0.04	0.11		0.09	0.02	0.06	0.08		0.009	0.01	0.003	0.02
Lung	0.15	0.005	0.09	0.10	0.14	0.08	0.05	0.09	0.34		0.009	0+	0.01	0.04
Kidney	0.05	0.12	0.11	0.09		0.12	0.12	0.09	0.25		0.01	0.009	0.02	0.09
Spleen	0.03		0.04	0.10		0.08	0.01	0.03			0.008	0	0	0.02
Submax	0.02	0.07	0.08	0.20		0.06	0.03				0.007		0.007	0.04
Testicles	0.005		0.03			0.008						0	0.008	0.02
Adrenals			0.07								0.002	0	0	0
Muscle	0.003		0.04				0.004				0.003	0.03	0.002	0.01
Heart	0.01	0.06	0.05				0.05				0.007	0	0.004	0.03
Bile	0.51	0.02	0.17								0.03	0.004		0.01
Diaphragm			0.04									0		0.02
Feces												0		0.06
Skin-fur*		0.03	0.10				0.004				0.009	0	0.006	0.04
Fur*		0.02	0.04								0	0	0.01	0.09

* Probable contamination.

ied, for example, thyroid, blood, urine, etc. In each of the other columns the per cent of the original dose of radioactive iodine per gram of the various tissues is recorded. Each vertical column represents the results on one rabbit. The tissues were taken at the times indicated following the intravenous administration of radioactive iodine.

Blood. The radioactive iodine is injected directly into the ear vein, yet at the end of 5 minutes, approximately 70 per cent of the injected material has been removed from the blood stream. The blood radio-iodine level falls during the 48 hours studied. However, there is a wide variation, e.g., for period over 12 hours, three determinations gave from 0.003 to 0.06 per cent of the original dose present per cubic centimeter of serum.

Urine. Radioactive iodine from the blood appears almost immediately in the urine. Two animals were sacrificed 5 minutes following the injection of the iodine. In one, none had reached the bladder urine but it had reached the kidney to the extent of 0.12 per cent of the injected dose per gram. In the other, the kidney contained 0.05 per cent per gram of the injected dose and the bladder urine 0.19 per cent per cubic centimeter. As much as 37 per cent of the injected dose may be excreted in urine in 9 hours.

Thyroid. The thyroid gland picks up a remarkable proportion of the dose. Five minutes following injection, the thyroid contains 1.2 per cent of the injected iodine per gram of thyroid tissue. Later the amount of radio-iodine in the thyroid increases (as much as 20 to 30 per cent of the dose). There is a considerable and unexplained variation in the percentage of the dose found in the thyroid.

Other tissues. In the other tissues examined, there was some indication of regularity in the percentage of the radio-iodine dose found per gram of moist tissue. Thus, lung and kidney usually contained relatively high percentages; liver, spleen, heart, bile, and submaxillary gland usually contained intermediate percentages; and muscle, skin, diaphragm and testicle contained low percentages of the dose. The low percentages reported for fur are probably the result of accidental contamination.

DISCUSSION. The high content of the marked or radioactive iodine found in the thyroid may be a function of two factors: 1, increase in iodine content of the tissue; and 2, an increase of radioactive iodine by exchange because of the high "radioactive iodine to non-radioactive iodine" ratio in the circulatory system as compared to the low "radioactive iodine to non-radioactive iodine" ratio in the thyroid immediately after injection. Hertz et al. (4) have observed that markedly hyperplastic thyroid glands concentrate approximately 0.6 per cent of the injected material in about 40 minutes, and that normal thyroids only concentrate in the vicinity of 0.1 per cent of the injected amounts in 10 to 50 minutes. In experiments reported here the normal thyroid concentrates various amounts, fluctuating between 0.29 to 1.23 per cent of the dose given within the first hour, which, in general, confirms the results of Hertz et al. for normal tissues.

SUMMARY

1. Following intravenous injection of radioactive iodine, there is an immediate distribution to the various tissues from the blood with considerable fluctuation in the relative amounts found in any organ at the various periods observed.

2. The thyroid takes up a relatively large percentage of the injected dose.

3. The radio iodine appears promptly in the urine. Up to 30 per cent of the dose may be excreted in 9 hours.

4. Lung and kidney tissue contain relatively high percentages of the radio iodine dose; liver, spleen, heart, bile and submaxillary gland contain intermediate amounts; and muscle, skin, diaphragm, and testicle have low percentages.

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THE MAGNITUDE AND TIME OF DEVELOPMENT OF THE COLLATERAL CIRCULATION IN OCCLUDED FEMORAL, CAROTID AND CORONARY ARTERIES[1]

RICHARD W. ECKSTEIN,[2] DONALD E. GREGG
AND WALTER H. PRITCHARD[3]

*From the Department of Medicine, Western Reserve University School of Medicine,
Cleveland, Ohio*

Although anatomical studies (1, 2) reveal that collateral connections establish themselves in various body regions following ligation of the main artery to that region, we have only a limited knowledge concerning the magnitude, time of development and pressure relations of such collaterals. Accordingly, experiments were designed to study these phenomena following acute and chronic ligation of femoral, carotid and coronary arteries.

METHODS. 1. *Femoral and carotid artery ligation.* In acute experiments dogs were anesthetized with sodium pentobarbital, chloralose, or morphine and ether, and the blood rendered non-coagulable with heparin and chlorazol fast pink or pontamine fast pink. The femoral artery was ligated in the upper third of the thigh and the carotid artery at the level of the thyroid cartilage. A cannula was placed in the main artery or in a side branch peripheral to the point of ligation. Retrograde flows were measured in a graduate or with a volume recorder, and retrograde pressures, together with aortic or femoral pressures were determined with Gregg (3) manometers. Measurements were made immediately and frequently during several hours after ligation. In some experiments blood pressure was altered with an aortic clamp, neosynephrine or augmented venous return. In other experiments, following an increase in retrograde pressure and flow, the ligature was removed and re-applied later. In chronic experiments dogs were operated aseptically initially and retrograde flows and pressures determined. At intervals varying from one day to many months the retrograde flows and pressures were again measured. In some cases the vein draining the region fed by the artery was also ligated, while in others the potential collateral sources were investigated.

2. *Coronary artery ligation.* Measurements of pressure and flow were

made in the same and in different dogs in a manner similar to that described for the femoral and carotid arteries, and for periods extending from a few seconds to seven days after ligation.

TABLE 1

EXP. NO.	DOG WEIGHT	DURATION OF OCCLUSION	BLOOD PRESSURE	RETRO-GRADE PRESSURE	FLOW	REMARKS
	kgm.				*cc./ min.*	
			Femoral (9 experiments)			
1a	13	15 seconds	186/135 (femoral)	20/20	10	
1b	13	90 minutes	195/138 (femoral)	98/93	50	
2	12	5 days	140/108 (femoral)	82/74	100	
3	14	250 days	212/165 (femoral)	160/125	200	
			Carotid (8 experiments)			
4	13	1 minute	96/82 (carotid)	66/62	16	
5		3 hours	103/96 (carotid)		110	
			Coronary (25 experiments)—left descendens			
7a	18	2 minutes	65 (mean carotid)		2.4	
7b	18	20 minutes	100 (mean carotid)		5.8	
7c	18	41 minutes	65 (mean carotid)		2.8	
7d	18	55 minutes	65 (mean carotid)		3.2	
8a	12	35 minutes	118/94 (carotid)	31/10	1.9	
8b	12	6 hours	98/74 (carotid)	31/7	2.5	
9a	19	13 minutes	123 (carotid)		3.4	
9b	19	48 hours	85 (mean carotid)		6.8	
10a	15	7 days	102/74 (carotid)	68/22	8.5	
10b	15	7 days	121/91 (carotid)	101/65	18	Aortic clamp
10c	15	7 days	132/106 (carotid)	104/72	24	Aortic clamp
11a	20	15 seconds	107/86 (carotid)		2	
11b	20	30 seconds	97/75 (carotid)		0.7	Circumflex clamped
11c	20	45 seconds	83/60 (carotid)		1.1	Circumflex released
			Left circumflex			
12a	13.5	6 days	134/106 (carotid)	100/54	9.4	Aortic clamp
12b	13.5	45 min. later	95 (mean carotid)		6.0	Control
12c	13.5	47 min. later	95 (mean carotid)		4.0	Left descendens and vein ligated

RESULTS. *Acute experiments.* Typical retrograde flows and pressures from carotid, femoral and coronary arteries at periods varying from one minute to six hours following ligation are shown in table 1. Immediately

after occlusion these values are small. However, within a minute carotid and femoral retrograde pressures and flows increase and continue to increase for hours (cf. expt. 1). In figure 1 (A and B), within 15 seconds following femoral ligation, the peripheral pressure drops from the control femoral pressure of 180/130 mm. Hg to 20/20 mm. Hg and the pulse disappears. However, within 30 seconds in C a definite pulse is present and the pressure is 39/37 mm. Hg. In subsequent records the pressure and pulse continue to rise, rapidly at first in D (after 12 minutes' occlusion) and then more slowly until after 90 minutes (E) the pressure is 98/93 mm. Hg. Such a pulse bears no resemblance to the central femoral pulse and its rise follows the latter by 0.03 second. With such an increase in retrograde pulse and pressure the retrograde flow rises from 10 cc. per minute in B to 50 cc. per minute in E. Once open, such collaterals tend to remain patent for in F the femoral ligature was removed for one hour. In G the ligature is replaced and the retrograde pressure drops only to 72 mm. Hg in contrast to the 20 mm. Hg following the first occlusion. Furthermore, a small pulse is present, and within 7 minutes the pressure of 98/94 mm. Hg in I is equal to that (98/92 mm. Hg) present 90 minutes after the initial occlusion.

Similar changes in retrograde pressure and flow occur following acute carotid artery occlusion, figure 1, (J, K, L, M) with the following differences: 1, the carotid peripheral pressure always has a sizable pulse; 2, its limit of rise is reached more quickly, and 3, the pulse is patterned after the central carotid pulse.

On the other hand, contrasting with the rapid collateral development in the femoral and carotid arteries, is the small initial and slow increase in collateral function after coronary artery occlusion. (See table 1 and fig. 2.) In measurements on 17 dogs in which the mean blood pressure varied from 60 to 117 mm. Hg and the weight from 7 to 27 kgm., the flow varied from 0.5 to 5.8 cc. per minute, with only 4 of the values below 1 cc. per minute. With an average mean blood pressure of 84 mm. Hg, an average weight of 14.6 kgm., the average retrograde flow is 2.2 cc. per minute. The high flow is 5.8 cc. per minute with a weight of 18 kgm. and a blood pressure of 103 mm. Hg. The low flow is 0.5 cc. per minute with a weight of 10.5 kgm. and a blood pressure of 112 mm. Hg.

As evident in table 1, experiment 7, these values may slowly increase in any one heart during the first hour after occlusion. Figure 2 (A) was taken a few minutes after occlusion of the left descendens. The chest was then closed and normal respiration restored for 6 hours, at which time (fig. 2, B), in spite of considerable fall in aortic blood pressure and a constant peripheral coronary pressure, the flow increased slightly from 1.9 to 2.5 cc. per minute.

Chronic experiments. Typical results of retrograde flow and pressure
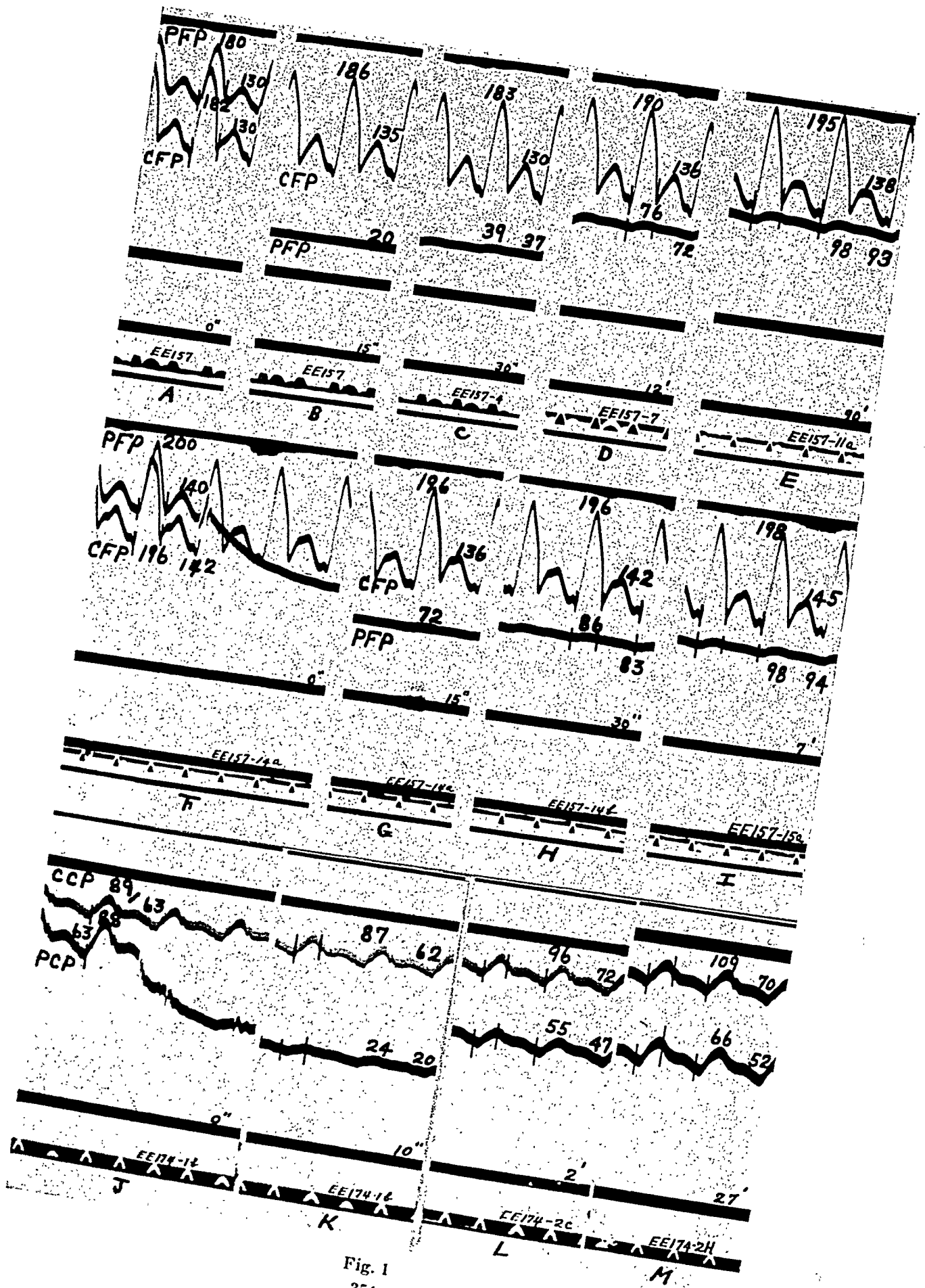
Fig. 1
354

are presented in table 1, and figures 2 and 3. After a few days of femoral and carotid artery occlusion the retrograde pulse and flow are large, but not much greater than after a few hours. However, after long-sustained occlusion the femoral retrograde pressure and flow become very large and the contour of the pulse resembles that of the central femoral pulse. In figure 3 A, after 250 days of occlusion, the retrograde pulse is 160/125 mm. Hg and the flow is 200 cc. per minute. Similar changes occur in the chronically occluded carotid artery (fig. 3, B, occluded 152 days). In both pulses, however, a considerable time lag is still present.
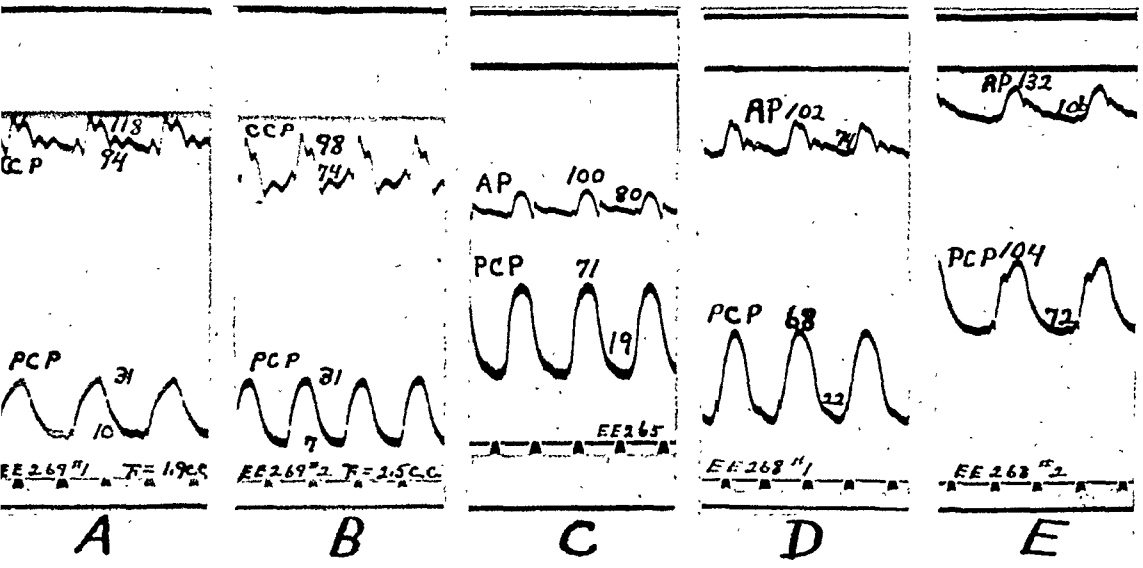
Fig. 2. Records showing changes in peripheral coronary pressure at different times after coronary occlusion. A, immediately after occlusion of the descendens. B, 6 hours later in the same dog. C, 48 hours after occlusion of descendens. D, 7 days after occlusion of the descendens. E, same, except aorta clamped. PCP, peripheral coronary pressure. CCP, central carotid pressure. Time, ⅕ second.

Within 48 hours the coronary collaterals are also further extended. For example, in one dog in experiment 9 of table 1 the retrograde left descendens coronary artery flow increases, after 48 hours of occlusion, from the initial value of 3.4 cc. per minute at a mean blood pressure of 123 mm. Hg to 6.8 cc. per minute at a mean blood pressure of 85 mm. Hg. See

Fig. 1. Records showing development of retrograde pressure in acutely occluded femoral and carotid arteries. A, control for B, C, D and E, taken at varying times up to 90 minutes after femoral ligation. F, record taken one hour after femoral release in E and serving as control for femoral re-ligation in G, H, I. J, control for records K, L, M taken at varying times after carotid ligation. PFP, peripheral femoral pressure. CFP, central femoral pressure. PCP, peripheral carotid pressure. CCP, central carotid pressure. Time, ⅕ second.
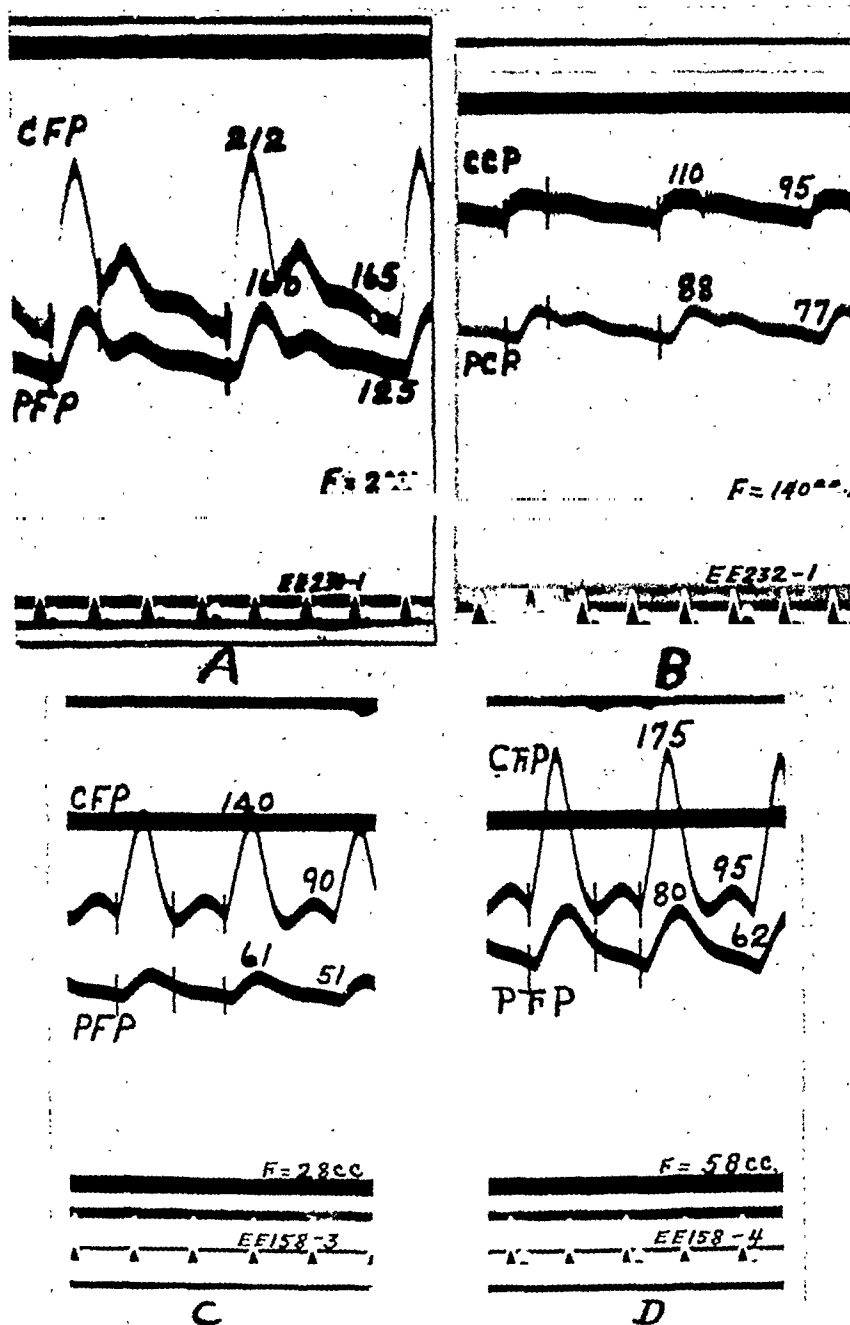
Fig. 3. Records illustrating high peripheral pulses after long continued carotid and femoral occlusion. A, after femoral occlusion for 250 days. B, carotid occlusion for 152 days. C, femoral occlusion for 90 minutes, and also serving as control for D in which venous return was increased. Letters and time same as in figure 1.

figure 2 C for records of left descendens peripheral coronary artery pressure after 2 days. These collaterals are still further augmented after 1 week, for, in table 1, experiment 10 (b) the left descendens artery has

been ligated for 7 days and the flow of 18 cc. per minute, with a mean blood pressure of 105 mm. Hg is greatly augmented over the highest acute value obtained in 17 dogs of 5.8 cc. per minute with a mean blood pressure of 103 mm. Hg.   Such an early increase in collateral function has also been demonstrated for the circumflex artery (table 1, expt. 12).   In spite of a
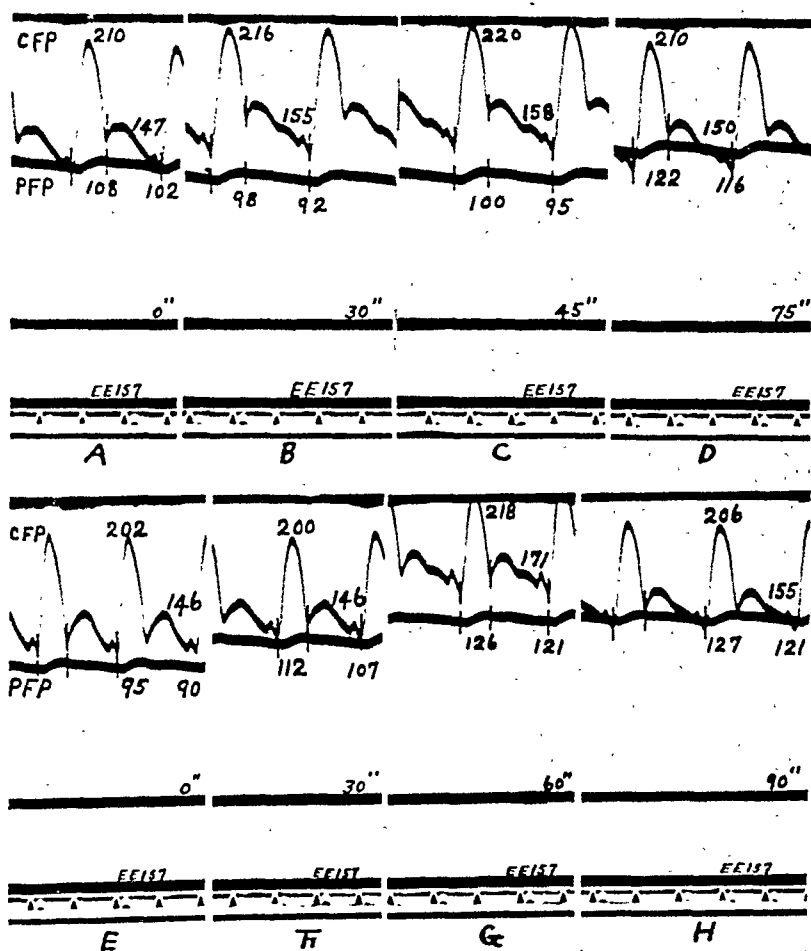
Fig. 4. Records showing dual effects of neosynephrine injection on the retrograde pressure in the acutely occluded femoral artery. A, control for B, C, D in which neosynephrine reduces the peripheral pressure. E, control for F, G, H in which neosynephrine increases the retrograde pressure.

rise in retrograde pressure and flow most of the myocardial area normally fed by an occluded coronary artery does not contract.   However, within a minimum of 7 weeks such ischemic areas do contract and values for retrograde pressure and flow are still further augmented (4).

Responses of collaterals to different circulatory conditions.   The effects of venous ligation, increased venous return, augmented blood pressure and neosynephrine on retrograde pressure and flow were studied.

Following ligation of both the femoral artery and vein in these experiments there is an increase in retrograde pressure and flow (record not shown). Similar results were found by other investigators (2). Increased venous return and elevated blood pressure likewise augment both the retrograde pressure and flow in the acutely and chronically occluded femoral and carotid arteries. In figure 3 C the femoral artery has been ligated for 90 minutes and the peripheral pulse is constant. Following an infusion of Locke's solution the retrograde pressure rises from 61/51 to 82/62 mm. Hg and the retrograde flow increases from 28 to 58 cc. per minute. Although neosynephrine usually increases both the retrograde pressure and flow, figure 4 shows that it may have a dual effect. The upper curves show an initial decrease of retrograde pressure in spite of a slight increase in aortic blood pressure, following intravenous neosynephrine. The lower curves show the usual effect. Following coronary sinus ligation both the retrograde coronary artery pressure and flow are increased (3, 5). Likewise, augmented venous return and increased blood pressure increase the retrograde coronary artery pressure and flow in the acutely and chronically occluded artery. (See fig. 2 (D and E) and table 1, expts. 10 and 7.)

Finally, the source of the retrograde flow was investigated by measuring the resulting changes in such flow following temporary occlusion of potential sources. As indicated in table 1, experiment 11, the retrograde flow from the acutely occluded left descendens artery decreased markedly following occlusion of the circumflex artery. After the circumflex artery was again released the retrograde flow increased in spite of a further fall in blood pressure. Similarly, the retrograde flow from the chronically occluded circumflex artery decreased by 33 per cent following occlusion of the left descendens artery (expt. 12).

DISCUSSION. The general trend of collateral extension in the three typical arteries is summarized in schematic form in figure 5. In the occluded femoral artery the pulse disappears, the flow drops to a very low value, to be replaced within a few seconds by a rapidly rising pulse and flow during a period of hours, and by a more slowly rising pulse and flow during days and weeks. The carotid artery behaves similarly except that a pulse is never lost, the retrograde pressure and flow do not sink to such low levels and more quickly approach their original value and contour. In the coronary artery, however, the retrograde flow is at first small and remains so for hours; the pulse may be smaller or larger than before occlusion, but is always at a lower diastolic level. The diastolic flow and pressure gradually rise to sizable values within a few days (2-6); the pulse with the same or different contour may have the same, smaller or greater magnitude.

The above indicates that the rate of collateral development in various

body regions is influenced to a different extent by various factors. The mechanisms for the collateral reactions in figure 5 are 1, the opening of preëxisting but non-functioning collaterals by a combination of differential pressure, metabolites, and nerve action, and 2, the formation of new collaterals.

In the peripheral femoral and carotid arteries the existence of the initial rapid rise of the peripheral pressure and flow immediately after occlusion is evidence that the collaterals involved at this stage preëxist. The main initial impetus to their opening is presumably the augmented differential pressure, since 1, the collaterals function within a few seconds after occlu-
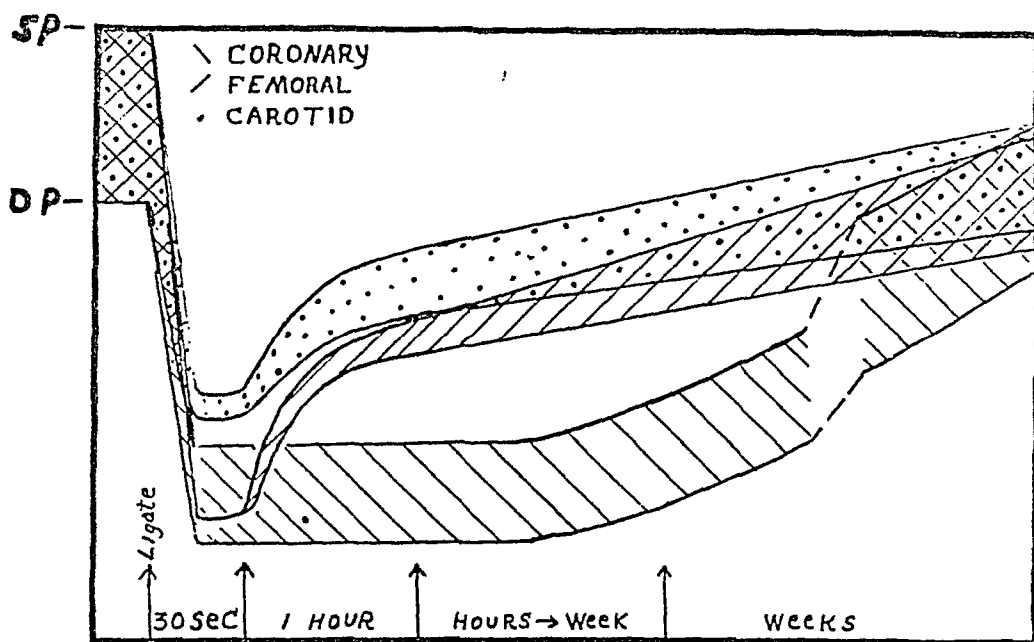
Fig. 5. Schematic drawing indicating the degree of collateral extension in the carotid, femoral and coronary arteries as evidenced by the retrograde flow, diastolic pressure and pulse. *SP*, systolic pressure. *D.P.*, diastolic pressure and flow.

sion; 2, the differential pressures are extremely high (166 and 63 mm. Hg respectively in fig. 1, B and K), and 3, procedures which increase further mechanically the differential pressure such as femoral vein ligation and elevation of systemic blood pressure (in the case of the carotid) give marked elevation of the peripheral pressure and flow.

The reason for the failure of the peripheral flow in an occluded coronary artery to increase for a number of hours and then only slowly in contradistinction to the femoral and carotid arteries is not known. Apparently the differential pressure is adequate and may approximate that in the carotid and femoral, while that in the right coronary may be even higher. For example, in figure 2 A the differential pressure averaged about 90 mm.

Hg, and yet after 35 minutes the retrograde flow was only 1.9 cc. per minute.

Following coronary artery occlusion the peripheral coronary diastolic pressure rises within 2 to 6 days and may, as in the case of the carotid and femoral arteries, be regarded as definite evidence of collateral extension. However, in contradistinction to the femoral and carotid arteries, the sizable pulse which persists after occlusion may show little or no increase as collaterals open. In other words, the pulse pressure here is not necessarily an index of collateral extension, but rather the resultant of various factors tending to make the pulse larger or smaller. The increased functioning of many small, deeply embedded coronary collaterals promotes the diastolic filling of the coronary bed and thus increases the systolic pressure and pulse. However, large superficial collaterals may acquire the central coronary pulse which is usually smaller than the peripheral coronary pulse.

Previous reports indicated that shortly after occlusion of the descending artery the retrograde flow ranges from 0.5 cc. (4) to 1.5 cc. (6) per minute. In the present experiments the retrograde flow from the acutely occluded left descendens artery is larger, with a range of 0.5 to 5.8 cc. per minute. The use of morphine and ether for anesthesia may explain the larger back flows reported here. Previous work (7) based on the small decline in peripheral coronary artery pressure following clamping of potential sources, was interpreted to mean that the other coronary arteries were not the source of the retrograde flow. The present studies confirm the magnitude of the decline of peripheral coronary artery pressure following ligation of potential sources of collateral flow, but actual measurements of retrograde flows show that, like coronary arteries occluded for many weeks (4), the major source of retrograde flow may be other coronary arteries. The most probable explanation for failure of the peripheral coronary pressure to decrease is that after occlusion the coronary bed is so poorly filled during diastole that further reduction in diastolic filling by ligating a coronary collateral is insufficient to affect the diastolic pressure and hence the pulse pressure.

SUMMARY

The time rate of collateral development has been studied in the femoral, carotid and coronary arteries, by means of the retrograde pressure and flow.

In the femoral artery immediately after occlusion the retrograde pressure and flow approximate 20 mm. Hg and 10 to 15 cc. per minute, respectively. Immediately, a small pulse appears and these values rise rapidly for a few hours and then more slowly for days and weeks until pulse and flow may approach those existing in the other intact femoral artery.

In the carotid artery the initial retrograde pressure and flow are somewhat greater than in the femoral artery; a pulse is always present and these approach more quickly the normal for the other carotid.

Following coronary occlusion the retrograde flow is nearly constant at values between 0.5 to 5.8 cc. per minute and then after some hours it, together with the peripheral diastolic pressure, increases very slowly to obtain sizable values in a week or so. As in coronary arteries occluded for many weeks, the major source of such retrograde flow may be the other coronary arteries.

Evidence is given to indicate that initial retrograde flow is due to increased differential pressure opening preëxisting collaterals. The mechanism for further collateral extension is not known.

In addition to the flow, the rise of the peripheral diastolic pressure in all the arteries and the peripheral pulse in femoral and carotid may serve as an index of collateral extension.

The peripheral pulse and retrograde flow are elevated following increased venous return and augmented blood pressure, but may be either increased or decreased by neosynephrine.

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THE PATTERN OF SERUM PROTEINS DURING ACCELERATED GROWTH[1]

E. S. ZAWADZKI AND ARTHUR H. SMITH

From the Department of Physiological Chemistry, Wayne University, College of Medicine, Detroit

Pursuing the general program of study of the chemical changes incident to growth, Smith and Gross (1937) have described changes occurring in the blood of albino rats following retarded growth as well as during unusually rapid development. It was demonstrated that animals in which increase in body weight had been restricted through lack of either the essential amino acid, lysine or of food energy, show an unusually rapid maturation of the blood picture as judged by the change in number of erythrocytes, concentration of hemoglobin and change in proportion of reticulocytes. Furthermore, upon realimentation either with a qualitatively adequate diet or with one providing requisite energy, a more youthful blood picture again appears and the process of hematologic maturation is resumed paralleling the accelerated somatic development. Smith and Gross ascribed the early rapid maturation of the blood to the stabilization of the blood volume induced by restriction of growth; the return to the more youthful blood picture upon realimentation seemed to parallel the increasing demand for blood which results when growth is resumed. The authors suggested that the rapidly expanding vascular bed may be more readily filled with fluid than with formed elements so that the immaturity of the blood picture following realimentation is the result of dilution accompanying or preceding resumption of hematopoiesis. The present study was undertaken in order to determine the chemical nature of this diluting fluid (plasma) during restriction of growth and during accelerated growth following realimentation.

PROCEDURES. The experimental conditions established by Smith and Gross (1937) were reproduced as closely as possible and hemoglobin determinations and erythrocyte counts were used to confirm the earlier observations. The pre-experimental diet of all animals was somewhat

richer in protein, however, than in the earlier study (see table 1), and the period of restricted feeding was shortened from six to three weeks. The data of Smith and Gross suggested this economy and it was shown by preliminary experiments that the results were not affected by this change in procedure. It was also demonstrated that the most profound change in the blood picture occurs three days after realimentation of the animals previously maintained on the restricted diets.

Male albino rats weighing 45 ± 5 grams at weaning (21 days of age) were given an adequate diet (pre-experimental diet, table 1) and 200 mgm. of yeast daily; those animals reaching 110 ± 5 grams body weight in 15 ± 2 days were changed to the experimental rations described in table 1.

TABLE 1

Composition of diets

	PRE-EXPERI-MENTAL DIET	EXPERIMENTAL DIETS			
		Diet I	Diet II	Diet III	
Lactalbumin*............	25	18		36	The vitamin B complex
Gliadin**..............			18		was supplied as dried
Dextrin†...............	44	51	51	23	yeast‖ apart from the
Hydrogenated fat‡.......	22	22	22	23	experimental ration.
Cod liver oil............	5	5	5	10	The amounts given are
Salts§.................	4	4	4	8	noted in the text.

* Labco no. 15-42, Borden Company, New York; moisture 6.5 per cent, total nitrogen 11.2 per cent, ash 1.6 per cent.

** Made from wheat gluten; moisture 5.0 per cent, total nitrogen 13.3 per cent, ash 0.5 per cent.

† White commercial.

‡ Crisco.

§ Osborne and Mendel (1917).

‖ Northwestern Yeast Company.

They were so distributed as to place litter mates into each of the three experimental groups as far as possible. The animals were housed in individual cages and body weight and food intake for each animal were recorded. Group I, consisting of twenty animals, was allowed to grow normally, being fed an adequate diet. Nine animals, serving as weight controls, were bled at 36 ± 2 days of age and 110 ± 5 grams body weight and the serums obtained were analyzed; the rest of the animals, serving as age controls, were fed diet I ad libitum until killed at an age corresponding to that at which their mates in group II and III were killed. Group II received diet II ad libitum; ten animals were killed and analyses performed at the end of three weeks on this ration and ten were realimented for three days with diet I following the three weeks of diet II. Seventeen animals in group III served as calorie controls receiving diet III in such

quantity daily as to maintain their weight at the level of those in group II. Eight animals were used for determinations at the end of three weeks on this diet and nine were realimented three days more with diet I before being used in the determinations.

Diet I was adequate in all respects, whereas diet II only lacked a sufficient quantity of lysine to maintain body growth at any but the slowest rate, and diet III, although qualitatively adequate, was fed at a low level to prevent body growth. Animals on the pre-experimental diet received 200 mgm. of dried yeast daily, those on the experimental diets, 400 mgm., whereas 600 mgm. were fed animals being realimented or weighing more than 200 grams.

Tail blood was used for the weekly erythrocyte counts, and hemoglobin and total solids determinations, care being taken to obtain freely flowing blood but to prevent excessive loss. The hemoglobin determinations were made by the acid hematin method using a photolometer calibrated by the oxygen capacity method. Total solids were determined gravimetrically after drying in a vacuum oven (Gradwohl and Blaivas, 1920). Blood for the determination of the protein and non-protein nitrogen fractions was obtained from the abdominal aorta, with the animal under urethane anesthesia. The serum obtained after complete formation of the clot and centrifugation was used for the determination of total nitrogen, non-protein nitrogen, and serum albumin and globulin nitrogens. Non-protein nitrogen was determined upon serum after precipitation of the proteins by trichloracetic acid. The digest obtained by heating with sulfuric acid and selenium oxychloride was nesslerized and read in a photolometer. For the determination of total nitrogen, untreated serum digested with sulfuric acid, potassium sulfate and selenium oxychloride, was distilled in a micro-Kjeldahl apparatus, the ammonia caught in boric acid, and titrated with hydrochloric acid. Globulin was separated from the serum according to the method of Howe (1921) as modified by Robinson, Price and Hogden (1937; 1938) care being taken to prevent adsorption and loss of serum albumin; serum so treated permitted the determination of non-protein plus albumin nitrogens by the procedure described above for total serum nitrogen.

RESULTS. The experimental data are presented in graphic form in figures 1 and 2 and are averages of values obtained on all the animals in each group, inasmuch as animals studied in the winter differed in nowise from those in the summer.

The growth of the rats in the various groups compared favorably with that obtained by Smith and Gross (1937) and expected changes in erythrocyte and hemoglobin levels were obtained (see fig. 1).

The growth of the rats in group I was accompanied by a rise in the average hemoglobin concentration from 12.5 grams per 100 cc. of blood at

38 days of age to 14.2 at 58 days of age.   There was a concomitant rise in the average total blood solid concentration from 17.82 to 21.05 grams per 100 cc.   The animals on the restricted diets showed greater increases of the hemoglobin levels and somewhat lesser gains in the total solid levels of the blood.   Thus the lysine-deficient group showed an average hemoglobin concentration of 15.03 grams per 100 cc. of blood at 58 days of age and the calorie control group 15.13 grams at 59 days of age.   These groups presented average total blood solid concentrations of 19.84 and 20.73 grams per 100 cc. respectively, in contrast to the 21.05 grams per 100 cc. of the normal group at the same age.   On realimentation, as in the experi-
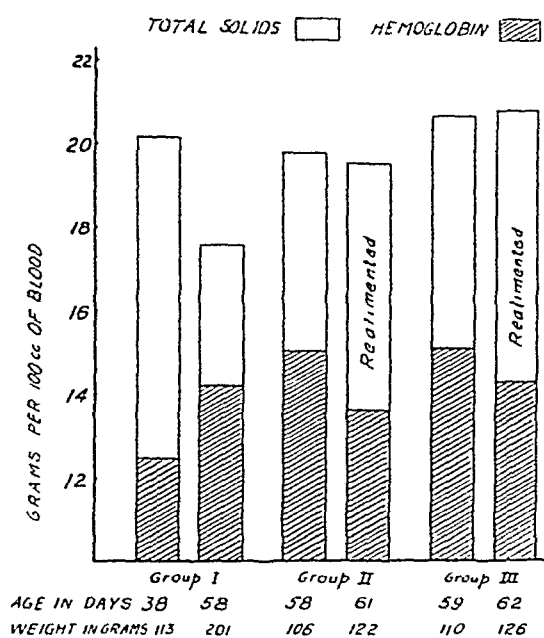
Fig. 1
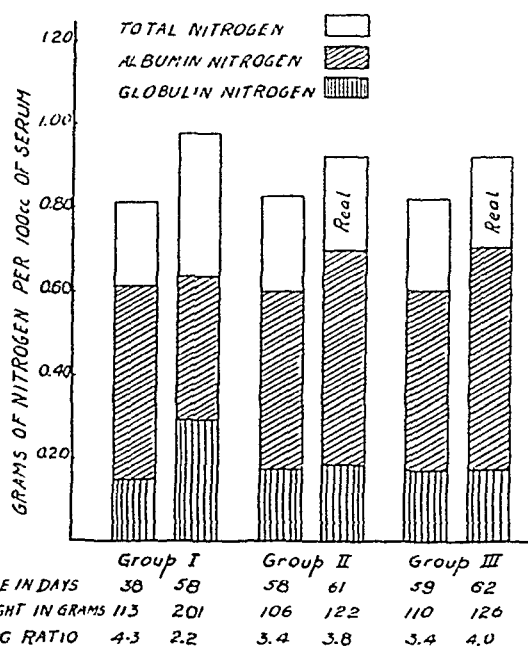
Fig. 2

Fig. 1. Mean values for total solids and hemoglobin concentrations
Fig. 2. Mean values for total nitrogen and protein partition of the serum

ence of Smith and Gross (1937) there was a fall in the hemoglobin levels, averaging 1.37 grams per 100 cc. in the case of the lysine deficient group and 0.77 gram in calorie control group.   The total blood solid concentration was not changed significantly in either group (19.84 grams per 100 cc. of blood before, and 19.55 grams after realimentation for the lysine-deficient animals and 20.73 as against 20.85 grams per 100 cc. of blood for the calorie control animals).   That the total blood solid concentration did not change upon realimentation is significant since it proves that simple hydration of the blood does not account for the fall in the hemoglobin and erythrocyte levels at this time.

The nitrogen fractions of the serum showed consistent relationships.

The non-protein nitrogen fractions throughout the experiment remain constant at 44 mgm. per 100 cc. of serum in all groups. The total nitrogen concentration in the normal group rose from 0.808 gram per 100 cc. at 38 days of age to 0.982 gram at 58 days of age, which compares favorably with results obtained by Swanson and Smith (1932) and Hatai (1918). This rise was due chiefly to an increase in the globulin fraction of the serum proteins (see fig. 2). The groups whose growth was restricted showed a barely perceptible rise in the total nitrogen of the serum, this being also due to a rise in the globulin fraction of the serum proteins. Upon realimentation, however, significant changes occurred. Both the lysine-deficient and calorie-control realimented groups showed a considerable rise in the total nitrogen concentration of the serum and in contrast to the situation existing in the normally growing animals, this rise was entirely due to an increase in the albumin nitrogen. During the three days of realimentation the level of total serum nitrogen increased from 0.829 gram per 100 cc. to 0.928 gram in the case of the lysine-deficient animals and from 0.827 to 0.921 gram per 100 cc. in the case of the calorie control animals. The albumin nitrogen concentration rose 0.091 gram from a level of 0.605 gram per 100 cc. of serum in the lysine-deficient realimented animals and 0.093 gram from a level of 0.609 gram in the case of the realimented calorie control group. These differences are statistically significant. It appears that the realimented animals although tending to approach the higher concentration of total serum nitrogen characteristic of normal animals of the same age, tend to revert to the characteristic blood picture of the younger animals of the same weight insofar as the protein nitrogen partition is concerned.

DISCUSSION. Smith and Gross (1937) concluded, from the decrease in erythrocyte count and the parallel drop in the concentration of hemoglobin, accompanying the accelerated growth during realimentation, that, under the stress of filling the rapidly expanding vascular bed, the ability of the organism to produce the fluid part of the blood exceeds the ability to manufacture red blood cells. Their observations on the reticulocyte response, however, indicated a prompt and vigorous hematopoiesis. Insofar as they postulated a dilution of the erythrocytes with a fluid not containing these formed elements, they apparently were correct. However, with the further information now available from the present study, it appears that the diluting fluid is plasma, which contains somewhat more protein than does the plasma of the restricted animals before realimentation.

Of particular significance from the point of view of hemodynamics is the shift in the relationship of albumin to globulin in the plasma of the experimental animals within three days of realimentation. The data on the increase in total nitrogen and that in albumin nitrogen show a high degree of statistical significance whereas the change in globulin nitrogen is

not outside the realm of chance. Melnick, Field and Parnall (1940) have indicated that the A/G ratio is highly reproducible and that the relations demonstrated by the salting-out technique "are real and indicative of the relative amounts of these two independent protein systems in native serum," although, in the light of the transformation of large to small "molecules" of serum proteins in the ultracentrifuge after dilution (McFarlane, 1935), they question the validity of predicting oncotic pressure in vivo from the data on the A/G ratio. However, in view of the demonstrated relationship between the albumin fraction of the serum proteins and the development of clinical and experimental edema on the one hand and the maintenance of blood volume on the other (see Weech, 1938–1939) the changes in the present study indicate the direction of the functional adaptation to the circulatory emergency created by the extremely rapid growth; not only is more fluid mobilized in the plasma but its composition is altered in the direction of relatively increased concentration of the serum protein fraction which is osmotically more active, namely, the albumin, and thus fluid is more efficiently held in the blood vessels. It is also obvious that, under the experimental conditions employed, little, if any, difference in the influence on the response to realimentation was demonstrated between retardation of growth through lack of dietary lysine or through low energy intake.

SUMMARY

Restriction of growth, as measured by body weight and produced either by deficiency of food energy or of the essential amino acid lysine, retards the usual progress of changes in the concentration of the serum proteins in the young albino rat. The usual rise in serum globulin is observed both in the normally growing animal and in the animal stunted by either of the nutritional deficiencies employed although to a much less extent in the latter. Realimentation of the stunted animals results in an increase of total nitrogen of the serum which is largely due to serum albumin fraction.

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HORMONAL INFLUENCES ON THE WEIGHT OF THE ADRENAL IN INANITION

MICHAEL G. MULINOS AND LEO POMERANTZ

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York

The two distinct responses of the adrenal glands to inanition are dependent upon whether the starvation is complete or the inanition is chronic in type (Jackson, 1919; Mulinos and Pomerantz, 1940). The purpose of the present report is *a*, to determine the size, weight and appearance of the adrenal glands during the two types of inanition; *b*, to study the effects of certain hormonal preparations and of implants of pituitary glands upon the adrenal glands during inanition; and *c*, to discuss the evidence that during chronic inanition the hypophysis plays an important part in the genesis of the loss of weight of the adrenal glands.

EXPERIMENTAL. One hundred and eleven female and 81 male rats, each about 200 grams in weight, were employed in this investigation (table 1). Twenty additional rats were used in the pituitary implant experiments detailed in table 2. Ninety hooded rats (both sexes) were used as pituitary donors.

The general procedure used was that described previously (Mulinos and Pomerantz, 1940). *Complete starvation* was instituted by the withdrawal of food and water for from 7 to 13 days. *Chronic inanition* was induced by reducing to half (about 6 grams daily) the usual amount of a complete food which had been consumed during a preliminary control period. The animals were weighed daily. When the hormonal preparations were used, they were injected subcutaneously each day. Estradiole benzoate (Progynon-B) was given to the female rats in doses of 2 to 10 R.U. and invariably resulted in estrus. Pregnancy urine extract (Follutein) was administered in doses of 4 to 10 R.U. to the female and 4 to 25 R.U. to the male rats; an alkaline anterior pituitary extract (growth factor) was given to female rats in doses of 5 to 10 growth units and testosterone proprionate to a male rat in doses of 0.5 mgm.[1] Seven female and 15 male rats were castrated and

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[1] Progynon-B and testosterone proprionate were kindly supplied by Dr. Erwin Schwenk of the Schering Corp. The Follutein and the alkaline extract of the anterior pituitary glands were generously furnished by Dr. H. S. Newcomer of E. R. Squibb and Sons.

divided into groups for further study of the effects upon the adrenal glands of inanition as compared with the fully fed controls.   Thirteen of the male rats received in addition testosterone proprionate in daily doses of 0.1 to 0.2 mgm. subcutaneously for six days.   The pituitary implants were taken from normal rats which were killed by decapitation.

At the termination of each experiment the rats were lightly anesthetized with ether and exsanguinated from the heart.   The organs were dissected and weighed on an analytical balance.   Rats that died during an experiment were discarded.

RESULTS.   The data are presented in table 1.   In *complete inanition* the weight of the adrenal glands is dependent upon the length of time of the starvation and upon the percentile loss in body weight.   At the end of the first week of starvation the adrenal glands are usually heavier, although they may be normal and at times reduced in weight.   As the period of starvation lengthens and the body weight loss becomes more severe the adrenal glands increase in weight.   When the starvation was allowed to continue until the rats became moribund the adrenal glands were invariably enlarged.   In contrast to the firm yellow glands of the normally fed controls, those of the starved rats were large and red with a cortex that was friable and congested with blood.   The adjacent peritoneal tissues were often edematous.

Chronic inanition induced in 9 male and 20 female adult rats, resulted in a progressive atrophy of the adrenal glands which was approximately proportional both to the duration of the inanition and to the percentile loss in body weight.   The adrenal glands of male rats are normally smaller than those of female rats of corresponding body weight or age.   In our series, the adrenal glands of the male rats averaged 28.1 mgm. in weight as compared with 47.4 mgm. for those of the female rats.   This difference is only in part accounted for by the fact that female rats weighing 200 grams are about 2 months older than males of equal weight.   These figures agree with the data of Donaldson (1924).   The loss of weight of the adrenal glands of the female rat was more marked (36 per cent) than the body weight loss (23 per cent); while in the male it was less (19 per cent) than the body weight loss (33 per cent).   During chronic inanition the female-male differential of adrenal weight was 7.2 mgm. as compared with a differential of 19.3 mgm. of the normally fed rats.   The adrenal glands of the female rat lost more in weight than those of the male rat, despite the fact that the males lost a greater percentage of their body weight.   The adrenal glands of chronically starved rats gain in weight when the rats are fully fed again, and attain normal weight when the loss in body weight has been recovered (table 1).   When the chronically underfed rat is in addition subjected to complete starvation, its atrophic adrenal glands gain in weight as already described under complete inani-

tion.   The small, firm and yellow glands become larger, softer and darker in color.

As for the normal adrenal gland (Selye, 1937), so during chronic inanition, noxious stimuli are capable of producing enlargement of the adrenal

TABLE 1

The weight of the rat adrenal glands as related to the type of inanition and as affected by hormonal injection or castration

STATE OF NUTRITION	DURA-TION	NUMBER AND SEX	SPECIAL PROCEDURE INSTITUTED	BODY WEIGHT CHANGE	ADRENAL GLAND WEIGHT
	days			*per cent*	*mgm.*
Fully fed controls		20 F	None		47.4 (39–52)
		4 F	Castrated 200 days	+33	33.6 (31–42)
Complete starvation	8.2	15 F	None	−37	54.3 (39–70)
	9.0	5 F	Inject. crude liver extract	−39	65.0 (45–79)
	8	10 F	Estrogen or P.U.	−37	56.6 (46–74)
	8	15 F	"Growth hormone"	−37	61.0 (50–75)
Chronic inanition	106	20 F	None	−23	30.0 (23–39)
	56	5 F	Refed	−18−+12	47.6 (46–49)
	81	5 F	Complete starvation terminally 4 days	−20−−40	35.6 (30–43)
	96	2 F	Spont. refusal of food until moribund	−38	54.0 (50–58)
	28–65	7 F	"Growth hormone" and/or P.U.	−27	36.0 (32–39)
	200	3 F	Castrated 225 days	−17	25.5 (22–28)
Fully fed controls		10 M	None		28.1 (25–31)
		5 M	Castrated*	+24	35.5 (32–39)
Complete starvation	7	7 M	None	−32†	34.1 (25–43)
Chronic inanition	72	9 M	None	−33	23.8 (21–27)
	65	3 M	Refed	−37−+14	30.6 (25–33)
	41–104	37 M	P.U. or testosterone	−29 (12–44)	24.8 (18–33)
	55	10 M	Castrated* 68 days	−34	23.6 (19.6–27)

* These groups include the rats which received injections of testosterone propionate daily for 6 days, without effect upon the adrenal glands.

† Rats weighed 277 grams before starvation.

glands.   Such stimuli may be in the form of injections of foreign materials, some disease process, a drug, etc.   Thus in almost all cases where non-adrenotropic hormonal preparations or tissue extracts were injected, or when the animal became ill and refused food, the glands were larger than the controls (table 1).   Obviously, the effects of illness and of such noxious

substances cannot be interpreted as being directly adrenotropic. Sufficiently noxious stimuli may cause an increase in the production of adrenotropic hormone by the hypophysis (Selye, 1937) or the adrenals may be rendered more sensitive to the adrenotropic hormone already present in in the animal even though this may be reduced in quantity.

An analysis of the conditions associated with the weight changes of the adrenal glands during inanition involved the following possibilities: a, the effect of inanition upon the glands directly; b, an alteration of pituitary adrenotropic hormone concentration in the blood due to hypophyseal inanition; and c, a diminution of gonadotropic and sex-hormone concentrations. The following experiments suggest that the adrenal weight changes from inanition are not due to a decrease in sex hormone concentration, and that the atrophy of the glands during *chronic* inanition is primarily due to a fall of the adrenotropic activity of the hypophysis.

The effect of gonadotropic hormone (P.U.); anterior pituitary extract; estradiole benzoate; testosterone proprionate; or of castration upon the weight of the adrenal glands during inanition. It has been shown that there is a deficiency of gonadotropic hormone during inanition. This deficiency is believed to be due to a failure of the gonadotropic function of the hypophysis (Mulinos et al., 1939; Werner, 1939). In consequence, the gonads would secrete fewer sex hormones. Because of the alleged functional relation between the gonads and the adrenal glands (Hall and Korenchevsky, 1938) it was necessary to determine the rôle played by these hormonal deficiencies in the origin of the adrenal gland weight changes during inanition.

Twenty-five female rats were starved completely and during this period were injected with estradiole benzoate, P.U., "growth hormone," or combinations of the latter two. These substances when injected in the doses already mentioned did not influence the gain in the weight of the adrenal glands which occurred from the complete starvation (table 1).

The hormones were also injected into 43 chronically underfed rats before and during the inanition, or after the inanition had progressed to a marked degree. The chronic inanition resulted in an atrophy of the adrenal glands despite the injection of these hormones. The potency of the hormones was gauged by the appearance of estrus and the increase of ovarian and adnexal weights in the female rats and by the increase in size and weight of the accessory sex organs in the males.

Castration of the female rat resulted in a reduction of the weight of the adrenal glands to 33.6 mgm. as compared with 47.4 mgm. for normal females (see also Hall, 1940). When chronic inanition was induced for 200 days in the castrated female rat the weight of the adrenal glands fell to 25.5 mgm. as compared with 30.1 mgm. for the chronically underfed uncastrated controls.

Castration of the male rat, in contrast to the female, resulted in adrenal

glands which weighed 35.3 mgm. as compared to 28.1 mgm. for normal rats (see also Hall and Korenchevsky, 1938). Chronic inanition for 55 days reduced the weight of the adrenal glands in both castrated and noncastrated male rats to 23.7 and 23.6 mgm. respectively. The injection of small doses of testosterone into castrated male rats had no effect upon

TABLE 2

The effect of pituitary gland implants upon the weight of the adrenal glands during chronic inanition

	CONTROL GROUPS				PITUITARY IMPLANT GROUPS							
							Adrenals		Ovaries		Thyroids	
Rat number	Body weight loss	Adrenals, weight	Ovaries, weight	Thyroids, weight	Rat number	Body weight loss	Weight	Per cent deviation from control average	Weight	Per cent deviation from control average	Weight	Per cent deviation from control average
Experiment I, February to July												
Underfed 151 days. No implants					Underfed 152 days. 8 pituitary implants from ♂ and ♀ donors							
	per cent	*mgm.*	*mgm.*	*mgm.*		*per cent*	*mgm.*		*mgm.*		*mgm.*	
43-1	21	30	25	14.0	44-1	25	38	+26	30	+20	13.5	−3
43-4	25	37	37	14.5	44-2	28	40	+33	39	+56	10.0	−28
43-5	30	26	13	13.0	44-3	38	40	+33	38	+52	19.0	+36
45-1	25	24	27	17.0	44-4	28	43	+43	38	+52	11.0	−21
45-3	32	33	22	11.0	44-5	37	32	+7	21	−16	13.0	−4
Av.	27	30.0	25.0	13.9		31	38.6	+29	33.2	+33	13.3	−4
Experiment II, August to November												
Underfed 114 days. 10 brain implants					Underfed 113 days. 10 pituitary implants from ♀ donors							
71-1	29	38.0	23.3	13.8	72-1	34	47.4	+40	37.8	+136	13.8	−4
71-2	32	29.8	15.6	16.7	72-2	37	49.7	+46	35.0	+119	10.3	−28
71-3	37	36.3	11.7	11.5	72-3	31	38.8	+14	33.2	+107	11.3	−21
71-4	36	32.5	19.1	16.8	72-4	35	50.0	+47	37.3	+133	12.0	−16
71-5	31	33.3	10.5	13.3	72-5	39	55.6	+64	20.0	+25	9.4	−34
Av.	33	33.9	16.0	14.4		35	48.3	+42	32.6	+104	11.4	−21

the weights of the adrenal glands of either the chronically underfed or the fully fed groups. The testosterone was given for only 6 days but proved sufficiently potent to cause growth of the accessory sex organs.

Since the responses of the adrenal glands to inanition occurred in spite of the fact that these hormones were supplied artificially, it may be con-

cluded that the gain in weight of the adrenal glands during complete inanition, and the atrophy during chronic inanition, were not due to any deficiency of the sex or growth hormones which may have occurred during the inanition.

The effect of pituitary gland implants upon the weight of the adrenal glands during chronic inanition. The following experiments demonstrate that chronic inanition depresses the adrenotropic function of the pituitary glands as well as the gonadotropic. The results are summarized in table 2.

Two groups of 5 female rats were underfed for about 5 months. Each of the rats of one group received 8 whole pituitary glands from normal female and male donors. The injections were made into the hind leg muscles over a period of 10 days. Three days after the last injection both this group and the control group were autopsied. The efficiency of the pituitary implants was judged by the effects upon the weights of the sex organs (table 2, expt. I). The experiment was repeated upon a second group, the controls of which received intramuscularly a piece of brain tissue from the donor rats. This was done in order to obviate the chance that infection or trauma contributed to increase the weight of the adrenal glands in the pituitary implant group. Each of these rats received 10 implants, 5 rats receiving hypophyses of female donors, and 5 a piece of brain tissue (table 2, expt. II). At autopsy there was no evidence of infection or local tissue reaction. Among the 10 rats of the pituitary implant group, the adrenal glands of but one rat (44-5) fell within the range of control weights; all others had increased in weight above the controls.

It is probable that the weight increase of the adrenal glands of the pituitary implant groups was due to the adrenotropic content of the implanted hypophysis. It is believed that the thyroid glands played no important part in this phenomenon because in the rats which received the hypophyseal implants the thyroid glands were no heavier than, and histologically did not differ from, the controls (compare Emory and Winter, 1934) (table 2).

The results of the pituitary implant experiments suggest that the atrophy of the adrenal glands which accompanies chronic inanition is due primarily to a relative diminution of the adrenotropic hormone in the blood rather than to the direct effects of the malnutrition upon the adrenal glands.

<div align="center">SUMMARY</div>

Complete starvation in adult rats resulted in an increase in the weight of the adrenal glands. In contrast, chronic underfeeding resulted in a loss of weight of the adrenal glands usually relatively greater than the loss in body weight. Underfed rats which were refed and allowed to attain their normal body weight, at autopsy revealed adrenals of normal weight.

During complete or chronic inanition the possible fall in the concentration of gonadotropic hormone (P.U.), "growth hormone," estrogen, or testosterone, is not a major factor in affecting the weight of the adrenals. When injected in small physiologically active doses, these hormones do not markedly alter the gain or loss of the adrenal weight of the animals.

Despite continued underfeeding, pituitary glands of normal rats implanted into chronically underfed female rats resulted in a gain in the weight of the atrophied adrenal glands. This indicates that the atrophy of the adrenal glands was not due directly to the malnutrition, but at least in part to an insufficient amount of adrenotropic hormone. It is suggested that in the chronically underfed rat, this insufficiency of adrenotropic hormone has its origin in the physiologically depressed function of the pituitary gland.

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THE EFFECT OF BILE ACIDS ON HEPATIC BLOOD FLOW

F. S. GRODINS, S. L. OSBORNE, A. C. IVY and LEON GOLDMAN

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

The relationship of hepatic blood flow to bile formation has not been extensively studied (1). Schwiegk (2) has observed that the choleresis produced by the intravenous injection of sodium dehydrocholate (Decholin-sodium) is associated with a 100 per cent increase in hepatic arterial blood flow and a variable effect on portal venous flow; apparently only one animal was injected once.

This work was undertaken to ascertain if Schwiegk's observation could be confirmed and, if so, whether other bile acids had a similar effect. The results would have a bearing on the broader question of the mechanism of choleresis.

EXPERIMENTAL METHODS. In order to measure the rate of blood flow, it was decided to use the direct current thermostromuhr introduced by Baldes and Herrick (3). Before using this method, however, it was necessary to conduct extensive preliminary experiments to determine whether or not this instrument was reliable in our hands.

We have made repeated calibrations on the same thermostromuhr unit, as well as on other units, both *in-vitro* and *in-vivo*. The calibrations *in-vitro* were made by the gravity perfusion method using different vessels, different temperatures, and different heating intensities. Fresh citrated dog's blood was used as the perfusing fluid. In the calibrations made *in-vivo* the unit was placed proximally on the femoral artery. Heparin or chlorazol fast pink was injected as an anticoagulant. The artery was then cannulated 10 to 12 cm. distal to the unit, all intervening branches being ligated. The dog was bled through the arterial cannula, the flows being timed and measured. The blood pressure was kept fairly constant by returning blood into the opposite femoral vein.

The results of calibration. The essential results obtained during the calibration of one unit are shown in figure 1. Curve 1 was obtained *in-vitro* at 25°C. with a heater current of 620 ma. and was fitted to 70 experimental points by the method of least squares. Seven different arteries (5 carotids and 2 hepatics) were used in obtaining the data and 2 months elapsed between the first and last calibrations. The standard

error of estimate for a single observation in the prediction of V from G is ± 15 cc. per minute. The average per cent error over the entire range is 7 per cent. In curve 2, the points designated X were obtained with a
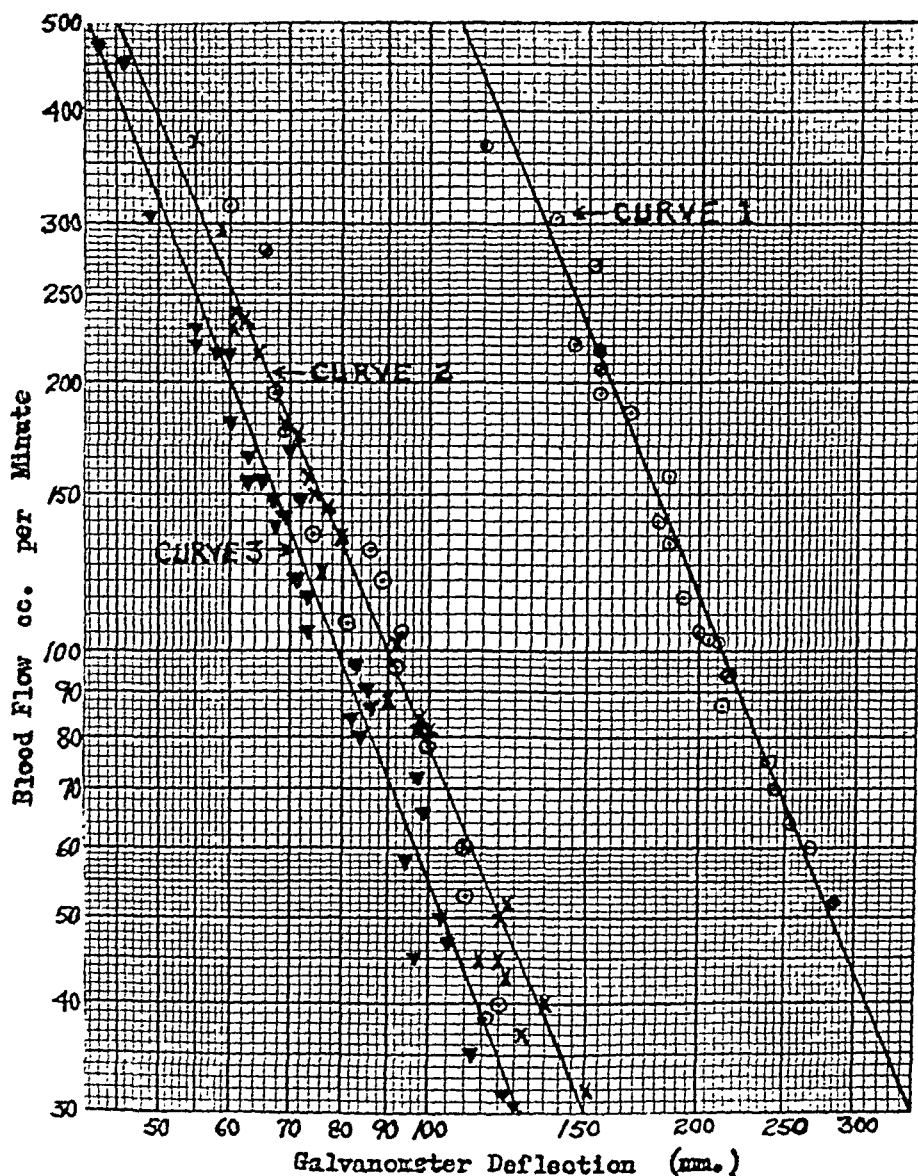
Fig. 1. Calibration curves for thermostromuhr unit E-4. Curve 1, *in-vitro*, 620 ma., 25°C. Curve 2, *in-vitro*, 400 ma., 25°C. indicated by X, 38°C., indicated by O. Curve 3, *in-vivo*, 400 ma., the data being obtained from 5 dogs.

heater current of 400 ma. *in-vitro* at 25°C. The curve was fitted to 60 experimental points by the method of least squares. Four different arteries (3 carotids, 1 hepatic) were used and 2.5 months elapsed between the first and last experiments. It will be noted that curves 1 and 2 have

the same slope and that the galvanometer deflections for any given flow are exactly proportional to the square of the respective heater currents. These facts give additional evidence for the reliability of the results. The standard error of estimate on this curve is ± 15 cc. per minute and the average per cent error is 7 per cent. The points designated by a dot within a circle on curve 2 represent data obtained *in-vitro* with a heater current of 400 ma. and a temperature of 38°C. The curve fitted to 51 such points is exactly superimposed on that obtained at 25°C. Curve 3 was obtained *in-vivo* with a heater current of 400 ma. and was fitted to 80 experimental points obtained from 5 different dogs. The standard error of estimate on this curve is ± 20 cc. per minute and the average per cent error is 10 per cent.

The preparation of the animals. In the bile acid studies acute experiments were performed on dogs under sodium pentobarbital anesthesia. Carotid blood pressure was recorded. The common bile duct was cannulated and the cystic duct ligated. The blood vessel to be studied was carefully dissected clean for a sufficient distance to allow application of the stromuhr unit. The unit was applied to the hepatic artery near the origin of the vessel as it arches in the border of the lesser omentum. It thus measured not only the blood going to the liver but also that going through the gastroduodenal branch. This latter vessel was not ligated because we wished to maintain normal circulation insofar as possible and to avoid any possibility of setting up eddy currents. The unit had to be adjusted carefully to avoid kinking of the vessel. On the superior mesenteric artery, the stromuhr was applied close to the origin of the vessel where it is free of branches. The correctness of the fit was tested by observing whether a steady zero point was maintained with no heat applied and also by noting whether *the galvanometer deflection with heat applied was proportional to the square of the heater current.* The dog remained "idle" for 30 minutes after completing all operative procedures. Then, after suitable control periods, recording blood pressure, bile flow, and blood flow, the effects of various bile acids as salts were determined.

The following salts were tested: sodium dehydrocholate[1] (Decholin-sodium) in 0.5 gram doses in 20 per cent solution; mixed triketocholanates[2] (Ketochol) in 0.25 gram doses on 10 per cent solution; pure sodium cholate[1] in 0.5 gram doses in 10 per cent solution; sodium taurocholate[3] in 0.5 gram doses in 10 per cent solution; and sodium glycocholate[4] in 0.5 gram doses in 10 per cent solution. All injections were given into the femoral vein and *at a rate that did not alter blood pressure, except as noted under results.*

[1] Riedel-de Haen.
[2] Searle and Company.
[3] Natural product (Riedel-de Haen) and a synthetic product (Searle).
[4] Synthetic (Searle).

TABLE 1

Effect of sodium dehydrocholate on hepatic arterial blood flow

DOG NUMBER	WEIGHT	WEIGHT LIVER	BLOOD FLOW		PER CENT CHANGE	CC./MIN./100 GM. LIVER (CONTROL)
			Control	Decholin		
	kgm.	grams	cc./min.	cc./min.		
1	22.7	680	215	275	+28	29
2	23.0	690	55	175	+218	11
			80	125	+56	
3	25.0	750	76	200	+163	20
			150	150	0	
			150	150	0	
4	25.0	750	185	265	+43	25
			190	210	+10	
5	20.0	600	150	200	+33	18
			105	175	+67	
			62	94	+51	
6	18.2	550	125	250	+100	18
			60	76	+27	
			43	58	+35	
7	9.1	275	74	90	+22	25
			66	80	+21	
			64	80	+25	
8	16.0	480	160	200	+25	34
			180	265	+47	
9	15.9	480	97	150	+54	21
			97	145	+50	
10	13.2	395	51	67	+31	19
			72	83	+15	
			76	97	+28	
			98	129	+32	
11	16.4	490	132	203	+54	30
			175	230	+31	
			160	245	+53	
12	22.7	680	255	480	+88	44
13	18.1	540	80	92	+15	19
			68	83	+22	

TABLE 1—*Concluded*

DOG NUMBER	WEIGHT	WEIGHT LIVER	BLOOD FLOW		PER CENT CHANGE	CC./MIN./100 GM. LIVER (CONTROL)
			Control	Decholin		
	kgm.	*grams*	*cc./min.*	*cc./min.*		
14	16.4	490	100	100	0	20
			100	100	0	
15	34	1000	245	310	+26	25
			180	315	+70	
			180	430	+139	
16	16	480	80	96	+20	17
			78	94	+20	
17	17.3	520	240	275	+15	32
			150	180	+20	
18	16	480	185	260	+37	50
19	17.7	530	125	200	+60	24
20	16	480	100	150	+50	21
			110	170	+54	
21	23.2	690	195	245	+26	26
			84	110	+31	
Averages			126	186	+48	

Photographic records[5] as well as direct readings of the deflection of the galvanometer were made.

RESULTS. *Hepatic arterial blood flow. A. Magnitude of normal flow.* The average normal flow through the hepatic artery of 28 dogs was 26 cc. per minute per 100 grams of liver with a range of 11 to 50 cc. per minute (table 1).

B. *Effect of intravenous sodium dehydrocholate.* We have performed a total of 46 experiments on 21 dogs (table 1). In 42 instances (20 dogs) an increase in blood flow was observed ranging from 15 to 218 per cent with an average of +48 per cent. The maximum increase in flow was reached within 5 minutes after the injection was started and the rate gradually decreased to its original level in about 15 or 20 minutes. In some animals, the increase lasted much longer (40 min.) and in others it was more transient (10 min.). In a few instances, the injection was given too rapidly and some fall in blood pressure occurred. In these cases, the

[5] The photographic records and other detailed data were demonstrated at the New Orleans Meeting of the American Physiological Society, March, 1940.

blood flow response was more rapid and tended to be greater than when the blood pressure did not change. An average bile flow increase of 700 per cent was observed. The maximum rate of bile flow was reached about 2 minutes after the maximum blood flow and the rate remained increased
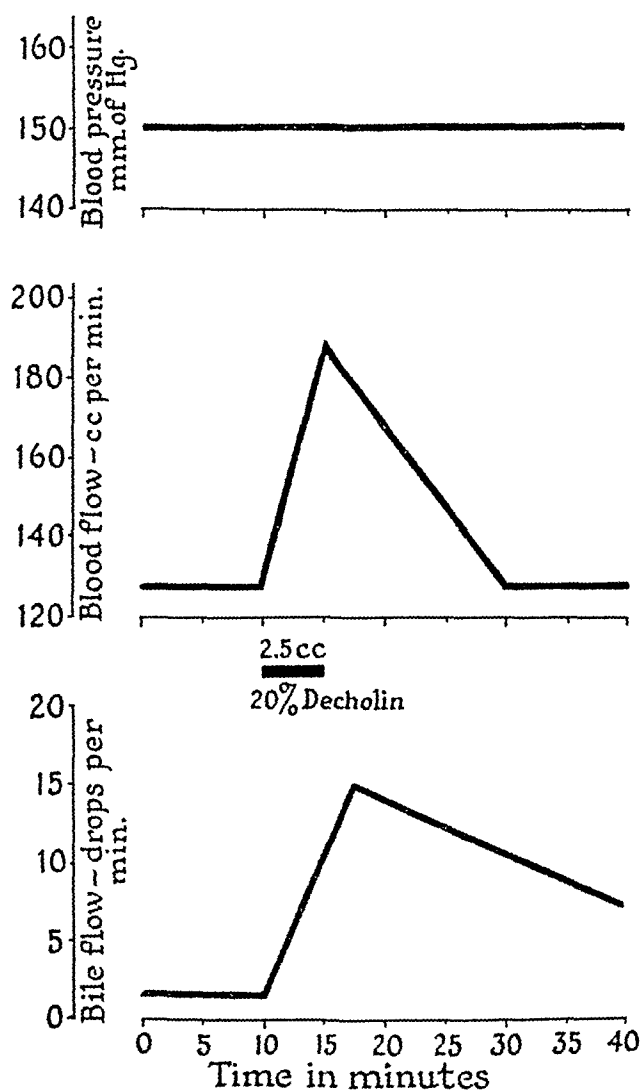
Fig. 2. Showing the effect of sodium dehydrocholate on hepatic arterial blood flow, bile secretion and blood pressure. The graphs represent the average values of 42 tests on 20 dogs.

for a longer period than did the blood flow. To conserve space the results on blood pressure, hepatic blood flow and bile flow were averaged and graphed, and are shown in figure 2.

In 4 experiments on 2 dogs, no change in blood flow was observed. In 2 of these cases, the secretory response was very slight. The other dog was totally refractory and showed neither an increase in bile flow nor in blood

flow. In 1 dog (not in table 1) whose postoperative condition was poor (blood pressure close to shock level), we injected the bile salt during a time when the blood pressure was gradually falling (to a low of 40 mm. of Hg) and observed a decrease of 50 per cent in hepatic arterial blood flow which paralleled the fall in systemic pressure. Nevertheless there was a marked increase in bile flow (800 per cent).

C. *Effect of mixed triketocholanates (ketochol).* Sixteen experiments were performed on 6 dogs (table 5). In 14 cases (6 dogs) an increase in blood flow ranging from 15 to 119 per cent with an average of 39 per cent was observed. The time relations and the bile flow responses were similar to those observed with sodium dehydrocholate.

In 2 experiments (2 dogs) no change in blood flow was observed, though an increase in bile flow occurred.

D. *Effect of sodium cholate.* Twenty-seven experiments on eleven dogs were performed (table 2). The results with this particular salt were quite variable. In 11 cases, no change in blood flow was observed. In 10 cases, a decrease in flow ranging from -16 to -55 per cent was observed (average—31 per cent). In 6 cases, an increase in flow from 19 to 86 per cent (average—47 per cent) was noted. There did not seem to be any correlation between the blood flow response and the degree of choleresis produced. In all but one dog sodium dehydrocholate was also injected and this salt produced an increase in blood flow in all except one animal. The latter was totally refractory to any bile salt both in regard to blood flow and bile flow. In any given dog in which sodium cholate produced an increased blood flow, sodium dehydrocholate produced a greater increase except in one case.

E. *Effect of conjugated cholates* (table 5). In 9 tests on 5 animals, *sodium taurocholate* produced a decrease in hepatic arterial flow in 6 cases. The average decrease was 16.5 per cent. In 3 instances no change occurred. *Sodium glycocholate* produced a decrease in hepatic arterial flow in 4 of 6 tests on 5 dogs. No change occurred in 2 cases.

Portal venous flow. A. *Average normal flow.* The average normal flow in 6 dogs was 18 cc. per kilo per minute, or 60 cc. per minute per 100 grams of liver.

B. *Effect of sodium dehydrocholate* (table 3). Fifteen experiments on 6 dogs were performed. In 8 cases (4 dogs) a decrease in portal blood flow was observed ranging from 10 to 44 per cent with an average of 29 per cent. In 5 experiments (3 dogs) an initial decrease in flow followed by an increase was observed. The initial decrease ranged from 14 to 43 per cent, averaging 23.4 per cent. The subsequent increase ranged from 21 to 46 per cent with a mean of 32.5 per cent. In 1 experiment, an increase in flow of 38 per cent was observed without any previous decrease. In 1 dog there was too much spontaneous variation to draw any conclusions.

Superior mesenteric artery. A. *Effect of intravenous sodium dehydro-cholate.* Fourteen experiments were performed on 7 dogs. In 10 cases

TABLE 2

Effect of sodium cholate on hepatic arterial flow

DOG NUMBER	WEIGHT	Na CHOLATE BLOOD FLOW		PER CENT CHANGE	DECHOLIN BLOOD FLOW		PER CENT CHANGE
		Control	Na Cholate		Control	Decholin	
	kgm.	*cc./min.*	*cc./min.*		*cc./min.*	*cc./min.*	
1	22.7	310	138	−55	255	480	+88
		150	94	−37			
2	18.2	110	110	0	80	92	+15
		98	98	0	68	83	+22
		68	97	+42			
3	16.3	96	96	0	100	100	0
		96	96	0	100	100	0
4	34	430	300	−30	245	310	+27
		310	310	0	180	315	+65
					180	275	+55
5	17.7	240	120	−50			
		215	400	+86			
		220	380	+73			
6	15.9	165	100	−39	80	96	+20
		150	110	−27	78	94	+20
		74	62	−16			
7	17.3	275	275	0	240	275	+15
		215	180	−16	150	180	+20
		150	150	0			
8	15.9	215	215	0			
		260	260	0	190	280	+37
9	17.7	105	125	+19	125	200	+60
		180	180	0			
10	14.5	127	170	+33	100	150	+50
		105	135	+28	110	170	+54
11	23.2	180	180	0	195	245	+26
		150	110	−27	84	110	+31
		105	86	−18			

(5 dogs) a decrease in blood flow was observed ranging from 8 to 49 per cent with an average of 22 per cent. In 2 cases (1 dog) an initial

decrease averaging 34 per cent was followed by a 22 per cent increase. In 2 cases no change occurred (table 4).

Effect of a slow continuous injection of sodium dehydrocholate into a mesenteric vein. Having determined that the relatively rapid injection of a concentrated sodium dehydrocholate solution into a peripheral vein produced a marked increase in hepatic arterial blood flow, the question arose, does the slow absorption of bile salts from the intestine such as occurs

TABLE 3

Effect of sodium dehydrocholate on portal venous flow

DOG NUMBER	WEIGHT	BLOOD FLOW		PER CENT CHANGE
		Control	Decholin	
	kgm.	cc./min.	cc./min.	
1	7.7	180	100	−44.5
		130	74	−43
			190	+46
		80	60	−25
			112	+40
2	9.1	82	74	−10
		100	60	−40
		63	52	−18
			80	+27
3	9.1	160	98	−39
		150	107	−29
		130	98	−25
4	10	70	60	−14
			90	+28
		52	43	−17
			63	+21
		55	76	+38
5	10.9	400	310	−22.5
		400	260	−35

normally following the flow of bile into the intestine in response to a meal also produce such a blood flow response?

With this in mind we have observed the effect of a slow, continuous injection of a dilute (1 per cent) solution of sodium dehydrocholate into a mesenteric vein. The animals were prepared as previously described. In addition, a small mesenteric vein was cannulated and connected with a drip apparatus. After a suitable control period, the venoclysis was started. The rate was so regulated that the animal received 0.5 gram of bile salt per hour (50 cc./hour). This is about the rate at which Schmidt, Beazell, Berman, Atkinson and Ivy (4) returned bile to the duodenum in their

studies on chronic biliary fistula dogs. The drip was continued for 1.5 hours and the dogs were observed for 0.5 to 1 hour after the infusion was stopped.

Four dogs were studied in this way (fig. 3). Two of these showed an increase in hepatic arterial blood flow which tended to return to the control level shortly before or shortly after the drip was stopped. The other two

TABLE 4

Effect of Na dehydrocholate on the blood flow in the superior mesenteric artery

DOG NUMBER	WEIGHT	BLOOD FLOW		PER CENT CHANGE
		Control	Decholin	
	kgm.	*cc./min.*	*cc./min.*	
1	20.4	245	225	−8
		250	180	−28
		175	157	−10
2	29.5	135	135	0
		150 {	160	+7
			125	−17
3	23	400	310	−22
4	15	300 {	235	−29
			410	+24
		285	145	−49
		215 {	130	−39
			255	+19
5	20	180	110	−39
		78	62	−20.5
6	18.2	340	290	−14.7
		185	130	−27
7	10	86	86	0

showed an increase in flow which was maintained for 40 to 50 minutes after the drip had been stopped.

In view of the fact that Walker, Schmidt, Elsom and Johnston (5) in their studies on renal blood flow employing the thermostromuhr technique have reported a tendency for the galvanometer to "drift" spontaneously toward the direction of an increased flow during the course of an experiment, we felt it important to have a control series of animals which were observed over a similar period of time. Four dogs were used as controls (fig. 3). Continuous observation of hepatic arterial blood flow was made over a period of from 1 to 2.5 hours. In 3 animals a slow mesenteric drip

of 0.9 per cent NaCl was used instead of the bile salt solution; in one animal nothing was done beyond observing the blood flow.

If the average curve of the control series is compared with the average curve of the treated series, a definite difference is noted. In none of the control animals was a persistent spontaneous "drift" of the galvanometer toward a more rapid flow noted. The few spontaneous increases which were observed were of relatively short duration and never lasted as long as those seen in the treated animals (1.5 to 2 hrs.). In one animal we noted a sudden swing of the galvanometer almost back to zero followed by as sudden a return to its former reading, all within 1.5 to 2 minutes. This type of deflection can be produced by a sudden temporary kinking of the vessel.
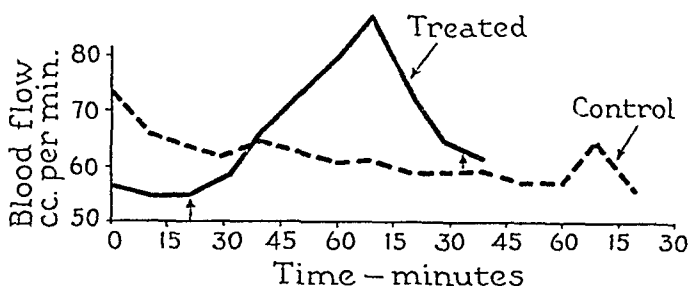
Fig. 3. Showing the effect of slow continuous injection of sodium dehydrocholate into a mesenteric vein on hepatic arterial blood flow; T, treated and C, control. The graphs represent the average values of 4 tests on 4 dogs.

DISCUSSION. Few data and instructions are available regarding the accuracy and the calibration of the thermostromuhr. The accuracy indicated by our results compares well with that (8 to 10 per cent) reported by others (6-9).

We were particularly interested in comparing our curve obtained *in-vitro* with that obtained *in-vivo*. When these curves are compared by statistical methods, which are not well adapted for comparing this type of data, it is found that there is no significant difference between them. In spite of this fact, however, the flows obtained *in-vivo* are consistently lower than those obtained *in-vitro* and one wonders whether this is a real difference due to conditional variations or whether it is due to the technical difficulties of obtaining calibrations *in-vivo*. From our experience we believe it is due to the latter. It is difficult to maintain a steady rate of flow in *in-vivo* calibrations whereas this is readily accomplished *in-vitro*. In the former case, the rate of flow tends to decrease with time and any lag in the galvanometer would give a reading too low for the observed flow. Another source of error is the possibility of turbulence produced by the ligation of the arterial branches.

Walker, Schmidt, Elsom and Johnston (5) believe that *in-vitro* calibra-

tions are invalid when applied to measurements on the experimental animal and indeed that even *in-vivo* calibrations cannot be applied from animal to animal. This possibility does not materially concern our conclusions, since we record a control flow, a test flow and a post-test flow, and compare the differences. We do not claim that the figures for the volume flows recorded here are absolute—the thermostromuhr is not a precision instrument. That they are relatively accurate, however, is indicated by the fact that our values for "normal" hepatic flows agree well with those obtained by direct methods (10–12).

For the purposes of comparison we had to be concerned principally with such items as uniformity of contact of the stromuhr with the vessel, turbulence and kinking. If the unit used is carefully selected as to size, and if certain tests are applied several times during the course of an experi-

TABLE 5

	HEPATIC ARTERY					PORTAL VEIN Na DEHYDROCHOL.	SUP. M. ARTERY Na DEHYDROCHOL.
	Na dehydrochol. (Decholin)	Mixed dehydrochol. (Ketochol)	Na cholate	Na tauroch.	Na glycochol.		
Number of dogs......	21	6	11	5	5	6	7
Number of tests......	46	16	27	9	6	15	14
Increased flow.... ...	42	14	6	0	0	1	0
No change...........	4	2	11	3	2	1	2
Decreased flow.......	0	0	10	6	4	8	10
Initial decrease to increase.............	0	0	0	0	0	5	2

ment we do not believe that the factor of contact should yield erroneous results. If the zero point of the galvanometer with no current passing through the heating unit fluctuates to any degree, one must assume that at times one of the thermojunctions has lost proper contact with the vessel wall. Another test is whether or not the galvanometer deflection is proportional to the square of the heater current at any given flow. The results of experiments in which these tests were unsatisfactory were disregarded in our work. In our experiments we were not concerned with very slow or rapid rates of flow. Our *in-vitro* and *in-vivo* tests, however, revealed that with the units we used very little reliance could be placed on flows of less than 20 cc. or greater than 400 cc. per minute (13, 14), and that the thickness of the wall of the vessels we used did not modify the results beyond the limits of error of the method.

The oxidized unconjugated bile salts produced a decided increase in hepatic arterial flow when injected intravenously. The fact that a marked choleresis was produced in 1 dog in which the hepatic arterial flow de-

creased along with a drop in systemic blood pressure indicates that sodium dehydrocholate can produce a choleresis independent of an increase in arterial flow. Usually, however, the choleresis produced by the oxidized cholates is associated with an increase in arterial blood flow. In this connection we have tested the effects of *cinchophen* on hepatic arterial flow because this substance is as effective an *hydrocholeretic* in the dog as sodium dehydrocholate (15). In doses of 50 mgm. per kilo intravenously this compound produced an increase of 47 per cent in hepatic arterial flow in each of two tests. This suggests that any true hydrocholeretic, a substance that increases the flow of bile and decreases both its content of solids and its viscosity, may increase hepatic arterial flow. Since an increase in arterial flow is observed when a slow injection of sodium dehydrocholate is given into a mesenteric vein to simulate the absorption of bile salts from the intestine during digestion, it may be that some of the therapeutic effects claimed for the oral administration of various oxidized bile salt preparations in hepatic insufficiencies are due in part to an increase in arterial flow to the liver.

It should be noted, however, that the total blood flow to the liver was not significantly increased by dehydrocholic acid and the unconjugated ketocholanates. As a rule, the increase in the total volume flow through the hepatic artery equaled the decrease in total volume flow through the portal vein. The hydrocholeresis was presumably associated with an increased oxygen supply to the liver. Whether this indicates that hydrocholeresis *per se* increases the oxygen demand of the hepatic cells or that such substances as ketocholanates and cinchophen are metabolized in such a manner as to increase oxygen demand is at present conjectural. The unconjugated and conjugated cholates, which are choleretic but not hydrocholeretic (16, 17), did not significantly affect hepatic arterial flow. Thus it is certain that unconjugated ketocholanates and cinchophen affect hepatic blood flow differently from cholates. And, in regard to glandular physiology, it is of special interest that the liver can secrete, in response to bile salts, an increased amount of secretion without the occurrence of a significant increase in total blood flow.

Although it was just indicated that ketocholanates and cinchophen may increase hepatic arterial flow by increasing oxygen demand, it is possible that they increase arterial flow by acting directly on the hepatic vasomotor nerves. If they decreased hepatic arteriolar tone, this would explain in part the hydrocholeresis, since it is known that section of the hepatic nerves has a hydrocholeretic effect (1) and such a change could explain mechanically the reciprocal changes in flow in the hepatic artery and in the superior mesenteric artery and portal vein (1, 18). If this is true, the hepatic vasomotor nerve endings or arterioles are specifically sensitive to unconjugated oxidized cholic acid, because we have found that ketochola-

nates are several times less potent in depressing blood pressure than the cholates (16).

The phenomenon of refractoriness to the intravenous injection of various bile salts which is present initially or develops during the course of an experiment is of much interest. In the course of various studies we have seen 17 cases of refractoriness in 170 dogs. The mechanism concerned is not clear (1).

SUMMARY AND CONCLUSIONS

1. The direct current thermostromuhr (Baldes and Herrick) has been tested on relatively large vessels and found to be accurate to about 15 per cent in a range of flows from 50 to 400 cc. per minute.

2. In 46 experiments on 21 dogs, sodium dehydrocholate given intravenously in 0.5 gram doses produced an increase in hepatic arterial blood flow in 42 instances. The increases ranged from 15 to 218 per cent, averaging 48 per cent. In 16 experiments on 6 dogs, the mixed triketocholanates in 0.25 gram doses produced an increase in hepatic arterial flow in 14 cases. The increase averaged 39 per cent and ranged from 15 to 119 per cent.

3. In 4 experiments on 4 dogs, sodium dehydrocholate given at the rate of 0.5 gram per hour into a mesenteric vein produced an increase in hepatic arterial flow in every case.

4. In two tests, *cinchophen* given intravenously in doses of 50 mgm. per kilo produced an increase of 47 per cent in hepatic arterial flow in both instances.

5. In 27 experiments on 11 dogs, sodium cholate in 0.5 gram doses produced no change in hepatic arterial flow in 11 cases, a decrease in 10, and an increase in 6.

6. Sodium taurocholate in 0.5 gram doses produced a decrease in hepatic arterial flow in 6 of 9 experiments on 5 dogs. Sodium glycocholate produced a decrease in 4 of 5 tests on 5 animals. The average decreases were 16.5 per cent and 13.7 per cent respectively.

7. In 15 experiments on 6 dogs, sodium dehydrocholate produced a decrease in portal venous flow in 8 cases, an initial decrease followed by an increase in 5, and an increase in 1. This salt produced a decrease in superior mesenteric arterial flow in 10 of 14 experiments on 7 dogs.

8. It is suggested that any true hydrocholeretic, as illustrated by sodium dehydrocholate, or the ketocholanic acids, and cinchophen, may increase hepatic arterial flow. On the other hand, substances such as the conjugated cholates which cause only a moderate increase in bile volume output with an increase in total solids without much change in viscosity do not produce this effect.

9. An increase in arterial flow to the liver is not essential for a choleretic response to bile salts.

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TRAINING IN HUMAN MUSCLES WORKING WITH AND WITHOUT BLOOD SUPPLY[1]

G. L. MAISON[2] AND A. G. BROEKER

From the Department of Physiology, St. Louis University School of Medicine

If a muscle is used daily to perform voluntary work its capacity for work will undergo marked enhancement by a process known as muscle training. Little is known of the mechanism by which the power of the muscle increases. It is possible that local changes in the muscle cells themselves may be responsible. Biochemical studies devoted to measuring changes in constituents of muscle as a result of training have not been very illuminating. It is possible that changes in the innervation of the muscle are responsible. This might involve the building of resistance to fatigue in the cells of the motor cortex or the anterior horn or a more economical usage of motor discharge. It is possible that changes in the blood supply may be responsible in increasing the oxygen available and facilitating the removal of metabolites. It seemed reasonable that removal of the influence of the circulation might yield significant information about this problem. Accordingly the training characteristics of muscle working in an ischemic condition (i.e., without blood supply) have been investigated.

METHOD. The experiments required that work be done by a single muscle; that the work be accurately measured; that the muscle be worked to a clearly defined end point each day; that the blood supply be interrupted during work in one muscle while its contralateral fellow worked with its blood supply intact as a control.

The muscle. The extensor digitorum communis muscle was selected for the tests. The arm was pronated on an ergograph with the major flexion crease of the palm at the edge. The fingers hung vertical at rest. The load was applied through a leather strap over the distal half of the middle phalanges of the fore, middle and ring fingers. Thus the fulcrum over which force was applied by the muscle was the metacarpophalangeal joint. The interphalangeal joints were kept extended at all times. This arrangement has the following advantages:

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1. No muscle other than the extensor of the digits can apply force to the work.

2. The landmarks are so definite that there is little chance that the manner of application of the hand to the ergograph can vary.

3. The muscle is not well trained in ordinary life activities.

The ergograph: A weight ergograph was constructed for the extensor of the fingers. Two automatic counters were so arranged that while one registered the total distance through which the load was moved, the other counted the movements made. Figure 1A shows the ergograph seen from the side with arm in place. Fore, middle and ring fingers are inserted in thong, q. The thong is attached by wire to a brass plate 6 by 1 by $\frac{1}{16}$ inch, n. This plate is held in position by a steel angle, o, which is slotted to receive the plate. A brass pin, p, is driven through the plate to act as a stop on the thong side of the angle. At its other end the plate makes connection with a chain, m, which passes over a pulley of $2\frac{3}{4}$ inches diameter, l. The
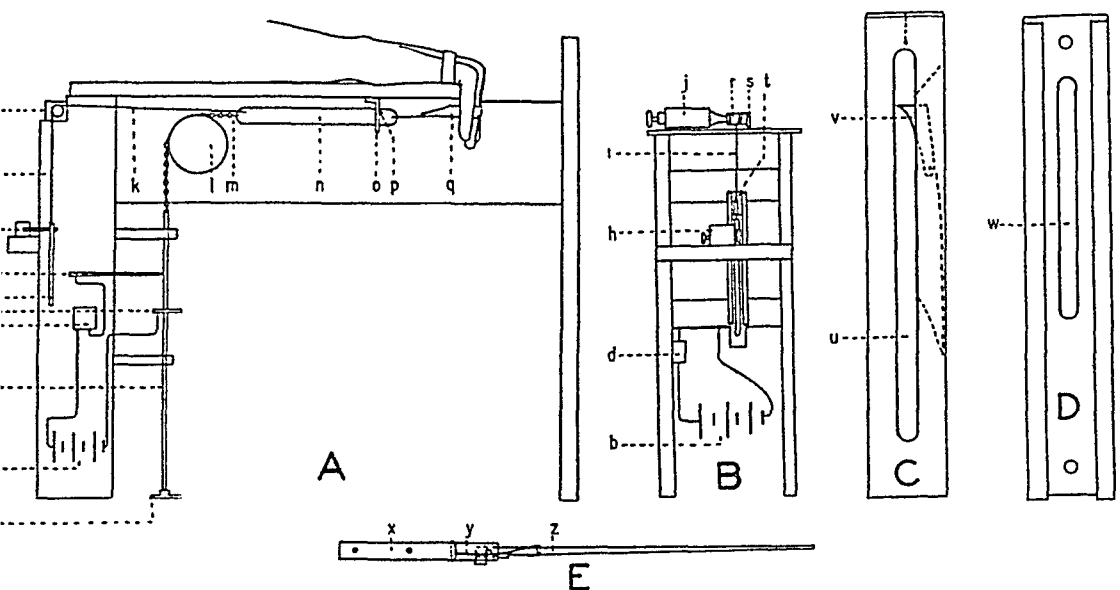
Fig. 1

chain after a vertical downward course of 6 inches attaches to a circular brass bar of $\frac{1}{4}$ inch diameter, c. The brass bar reaches to the floor and has threaded to it at its inferior end a circular brass plate $1\frac{1}{2}$ inches in diameter and $\frac{1}{8}$ inch thick which acts as a weight holder, a.

Just above the attachment of the chain on the plate, n, there is attached a cord, k, to operate the meters (h and j in fig. 1A and B). (Fig. 1B shows the ergograph from the back.) The cord passes to a spindle (r in fig. 1B) of 5 cm. circumference and $1\frac{1}{4}$ inches length. After several turns about the spindle the cord is anchored to the spindle. The spindle is united to the shaft of a Veeder reset rotary ratchet counter (no. L244) (j in fig. 1A and B) which registers 10 units for each rotation of the spindle. The other side of the spindle is supported by a brass bearing (s in fig. 1B) soldered to the plate on which the meter rests. This counter records the distance through which the weights are moved in 0.5 cm. unit.

The spindle also gives anchorage to a second cord (i in fig. 1A and B) which runs in opposite direction on the spindle to the first. After several turns this cord runs vertically downward to a brass rectangular bar 6 by 1 by $\frac{1}{8}$ inch (f in fig. 1A and in
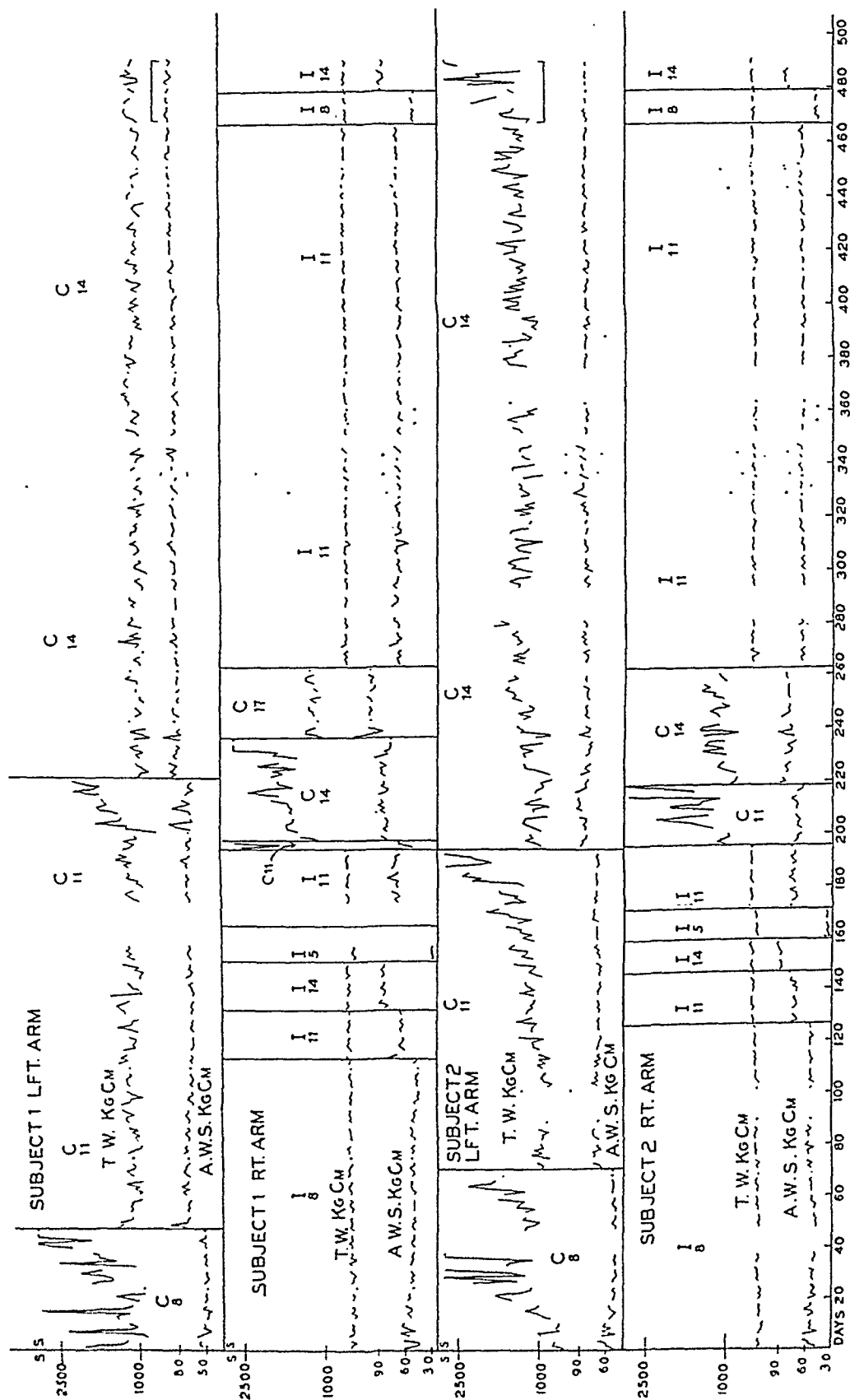
Fig. 2

detail as C in fig. 1). The bar runs in a metal track (t in fig. 1B and in detail as D in fig. 1). A Veeder reset ratchet single unit counter (no. ZD18) (h in fig. 1A and B) is mounted behind this metal track. Figure 1 C and D shows that both the slider bar and the track have a slot (u and w). The slider bar has a spring cog (v in fig. 1C) mounted so that it will move aside if an object approaches it in the slot from below but will hold fast if it is approached from above. The single unit counter has a lever attached to its shaft (see h in fig. 1A) which projects through the slots in the slider bar and track. When the machine is at rest the meter lever lies above the spring cog of the slider bar.

When the finger thong is pulled forward the weights are lifted and cord, k, is wound off the spindle. This motivates the distance counter. Simultaneously cord, i, is wound on the spindle from the slider bar which is thus pulled upward. As the bar moves upward the meter lever catches on the spring cog and is raised tripping the lower counter one unit. After the spring cog has risen about 3 cm., the lever, making an arc, has moved out far enough to slide off the spring cog and to fall back to its normal position. When the tension on the finger thong is released gravity returns the weights to rest, and acting on the slider bar pulls the bar back to rest. As the slider bar moves downward the spring cog is moved aside by the meter lever and again comes to rest below the lever. As the slider bar moves down it unwraps its cord, i, from the spindle and at the same time the other cord, k, is wrapped onto the spindle. Thus the weight of the slider bar is used as a counterpoise to return the meter cords to resting position after each stroke.

The inherent resistance to motion of the finger thong includes the weight of the load rack (a and c), the weight of the slider bar, f, which moves centimeter for centimeter with the load, and the resistance of the meters (h and j). This resistance was measured by running a cord from the finger thong over a pulley and vertically downward. Weights hung on this cord overcame the resistance of the ergograph. In construction the weight of the slider bar was so adjusted that the total inherent resistance of the machine was 500 grams. Weights were then made of lead in 300 gram units. Thus loads in 300 grams steps above 500 grams could be obtained.

The distance through which the load was moved was recorded by the counter in $\frac{1}{2}$ cm. units. This implies a maximum error of $\frac{1}{4}$ cm. in each stroke. Work was obtained as the product of load and distance.

Fig. 2. Plot of daily work of extensor digitorum communis muscle. Condition of muscle during work is indicated by C or I meaning "with blood supply" or "without blood supply" respectively, and by a numeral—8, 11, 14—meaning the load used in hundreds of grams. Hence $C8$ means muscle circulated working on 800 grams load. Rate of work with any given load is kept constant by making 1 stroke per second, each stroke about 6 cm. in length. $T.\ W.\ kg.\ cm.$ = total work in kgm. cm. $A.\ W.\ S.\ kg.\ cm.$ = average work per second in kgm. cm. $S\ S$ = steady state performance. Horizontal bracket in last twenty days of top muscle and third from top indicates that during this period the subject was undergoing an added exercise period of 15 minutes per day with a lighter load. Due to the marked reduction necessary certain points on graph are unlabelled: In top and 3rd graphs about 340th day the ischemic ability was tested with 1100 grams load for two days. In second and 4th graphs on three days near 340th day the ability with blood supply was tested with 1400 grams. Soon thereafter the ischemic ability with 800 grams was tested on two separate occasions. When work was done each day the points are connected. When a day elapsed without work, i.e., Sunday, the previous and succeeding points are unconnected.

Definition of fatigue. *Voluntary work without blood supply* produces a different fatigue curve than work with blood supply. The contraction height is well maintained and after a time ischemic pain is felt. Still later the ability to move is rather abruptly lost. Hence in these studies ischemic work was terminated when the subject could no longer lift the weight. Work was done at a rate of 60 contractions per minute.

Voluntary work with blood supply is accompanied by a gradual decline in contraction height. If the contraction height reaches a small enough level the muscle may be capable of continuing work in virtually a steady state. It is thus difficult to establish an end point unless the length of stroke is controlled. If the subject is required to make a minimum stroke of comfortable length the maintenance of a given rate of strokes affords a good end point. The subject continues making strokes of the set length until he can no longer make a stroke in the allotted time interval. Using the extensor digitorum communis adequately weighted at a rate of one contraction per second produces an obvious fatigue under this regime as shown by the marked associated movements, increase of blood pressure and flushing which accompany the last few strokes.

Fixation of stroke length was accomplished by arranging a buzzer to sound when the weights were raised to a given height. The subject was required to ring the buzzer with each stroke. Contacts for the buzzer are seen in figure 1A and B. A circular brass plate (*e* in fig. 1A) 2 inches diameter and $\frac{1}{32}$ inch thick was fixed by a set screw to the weight bearing rod, *c*. A flexible copper wire made connection from the plate to an ordinary door buzzer (*d* in fig. 1A and B). The other pole of the buzzer contacted a dry cell, *b*, whose second pole was connected to a spring lever, *g*, attached to the side board 6 cm. above the contact plate. The spring lever is seen as *E* in figure 1. One piece of brass sheet, *x*, $\frac{1}{2}$ by 3 inches is used as anchor. At its distal end it holds a loose rivet as axle for rotation of the lever, *z*, 6 x 0.010 x $\frac{1}{4}$ inch to $\frac{1}{2}$ inch which is crimped at its lower edge to prevent passage of the lever downward below horizontal. Upward motion of the lever is impeded by a short length of 0.015 inch music wire, *y*, which insures good contact between lever and plate when weights are raised. The effectiveness of this method of limiting the stroke is seen in the lines showing average work per second in figure 2. While the average rate of work with any given load is not constant it is relatively so.

Ischemia of the muscle was created by applying a sphygmomanometer cuff to the arm and inflating it instantaneously from a pressure reservoir of 5 liter capacity at a pressure of 240 mm. Hg. Vascular congestion was avoided by this rapid inflation.

Subjects. Twelve healthy male subjects were used. Eleven were graduate students between 22 and 28 years of age, 1 was a mechanician aged 45 (no. 1). All were in good health and all were accustomed to a semi-sedentary life. All had normal blood pressures.

The effect of daily ischemic work on ischemic ability. Nine untrained subjects exercised their extensor digitorum communis muscles to fatigue once each day, 6 days per week. The right arm was worked without blood supply, the left arm with blood supply. A load of 800 grams, a stroke greater than 6.0 cm. and a stroke rate of 60 per minute were employed. Four of the subjects worked for 4 months, the remaining 5 for $2\frac{1}{2}$ to 3 months. Table 1 shows average values of work done by the ischemic muscles at beginning and end of the experiments. A more

complete picture for 2 of the subjects is seen in the first 120 days of the right arm graphs in figure 2. Here one can see the daily variation of total work and of average work per second which reflects the constancy of the stroke distance.

The response of ischemic work capacity to daily work without blood supply was indicated in 6 of the 9 instances by a small increase. Cases 3, 4 and 7 showed almost no change. The others showed increases of from 6 to 25 per cent in the course of 10 to 17 weeks' work. The manner of rise was steady gradual increase in cases 1, 2, 6 and 8, and a sharp rise to near

TABLE 1

Effect of daily ischemic work on ischemic work capacity compared with speed of circulated training in each case

Sub-ject	RIGHT ARM ISCHEMIC DAILY AVERAGE WORK OF EXT. DIGIT. COMMUNIS WITH 800 GRAM LOAD IN KGM.CM.						LEFT ARM CIRCU-LATED. TRAINING REQUIRED TO PRODUCE STEADY STATE ABILITY IN WEEKS. 800 GRAM LOAD
	In first 3 weeks	In last 3 weeks		Diff.	Tested later after other experiments		
	Kgm.cm.	Weeks	Kgm. cm.		Weeks from start	Kgm. cm.	
				per cent			
1	495	15th–17th	527	+6.5	66th and 67th*	592	4 weeks
2	372	14th–16th	455	+22	66th and 67th*	508	5 weeks
3	485	12th–14th	498	+2.7			6 weeks
4	359	13th–15th	354	−1.4			17 weeks
5	434	9th–11th	541	+25	39th and 40th†	589	11 weeks
6	396	8th–10th	447	+13			Not complete at 11 weeks
7	384	10th–12th	405	+5.5			6 weeks
8	249	7th– 9th	278	+12			Little training at 10 weeks
9	433	7th– 9th	505	+17			5 weeks

* These subjects worked daily with various loads throughout this 16 month period.
† This subject worked 11 weeks then rested 13 weeks then worked 16 weeks.

terminal value within 4 weeks in cases 5 and 9. Subjects 1 and 2 continued working with different loads as shown by figure 2. Subject 5 after a 13 week absence returned to work with other loads. After 66 weeks' work by subject 1 and 2, and 39 weeks' work by subject 5, all 3 worked again without blood supply with an 800 gram load. Table 1 shows that a further increase had occurred. Subject 1 shows a total gain which amounts to about 1.5 per cent per month over the 16 month period.

During the same period described in table 1 the left arms of the 9 subjects were employed in work with blood supply intact. Starting out with 800 grams as the load most of the subjects soon reached steady-state ability as shown in the last column of table 1. There would seem to be little

correlation between speed of circulated training and degree of increase of ischemic work capacity.

Effect of load on ischemic work ability was tested in each of 4 subjects. The data are summarized as averages in table 2. Two of the subjects repeated the test, with the exception of the 500 gram load, 1 year after the original test. The figures show that work capacity was somewhat greater with heavier loads. In subjects 1 and 2 the year's training had apparently eliminated the advantage of the greater load. It is well known that with very light loads the efficiency of the muscle is reduced, since much effort is wasted in process of movement.

TABLE 2

Effect of load on ischemic work ability

Stroke rate of 60 per minute with all loads

SUB-JECT	CONDITION	LOAD							
		500 grams		800 grams		1100 grams		1400 grams	
		Trials*	Kgm. cm.	Trials*	Kgm. cm.	Trials*	Kgm. cm.	Trials*	Kgm. cm.
1	After 16 weeks' training	6	471	16	528	16	561	16	577
1	After 50 weeks' training			10	592	10	621	10	614
2	After 16 weeks' training	10	425	11	453	16	498	11	513
2	After 60 weeks' training			10	508	10	517	10	532
3	After 16 weeks' training	4	444	17	505	10	557	17	614
4	After 16 weeks' training	4	343	14	356	14	405	14	430

* Each trial was made on a separate day. The subjects worked only once each day.

The effect of load on ischemic pain accounts for the small number of observations on each subject with the lightest load (500 grams). Such exercise is intensely painful and subjects dislike doing it. The pain is less with greater loads though it comes on after fewer contractions. Finally most subjects showed fatigue without any pain with loads of 1700 grams or heavier. This phenomenon has been previously described by Katz and co-workers (2).

Effect of training with blood supply on ischemic work capacity. This was tested by having the subject work his arm without blood supply once daily until it was thought he was accustomed to the ergograph and his ability was known. In subjects 1 and 2 as shown by figure 2 this test was

made by the right arms after 7 months of daily ischemic work. Both arms of subjects 5, 10, 11 and 12 were tested after 2 to 4 weeks of daily ischemic work practise. Figure 3 shows the plot of the right arm of each subject.

Subjects 1 and 2. (See fig. 2, 170th to 280th day.) The right arms of subjects 1 and 2 were given a 2 month period of circulated training which
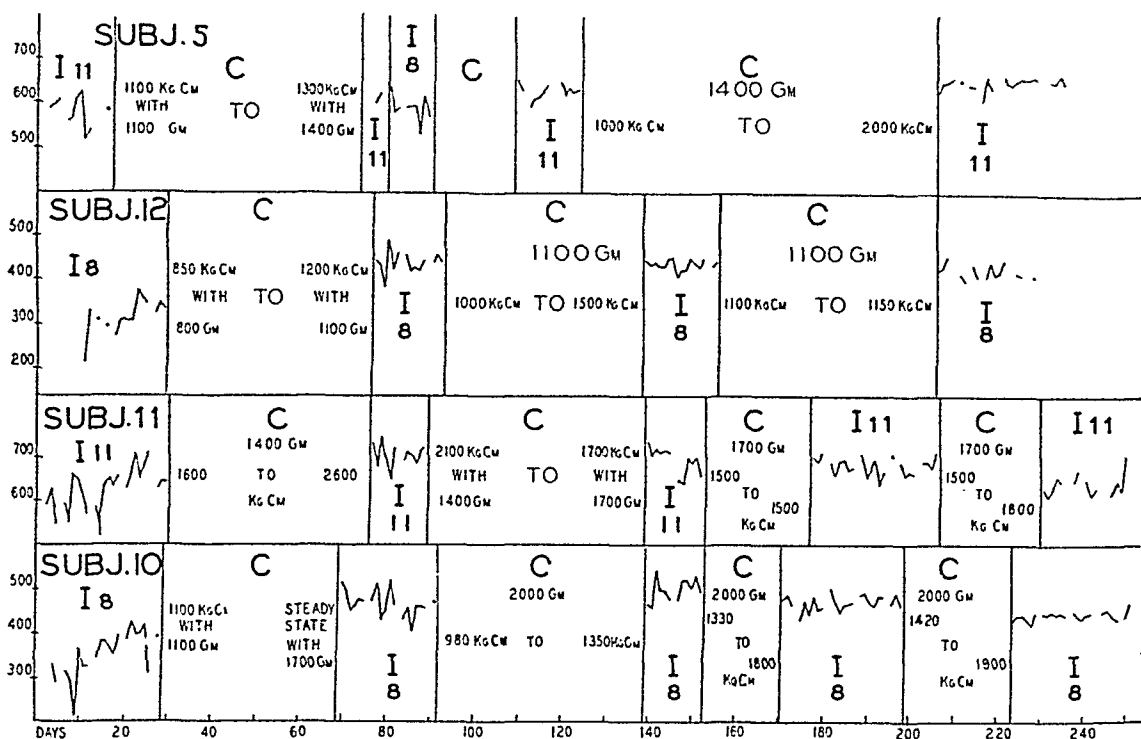
Fig. 3. Effect of circulated training on ischemic work capacity. Plot of daily work of extensor digitorum communis muscles. Total work of muscle without blood supply is plotted, load in hundreds of grams being indicated by the numeral—*I8* means ischemic work load 800 grams. *C* = circulated work to fatigue being done at this time. Work capacity and load at beginning and end of circulated training periods being shown by the numerals to indicate effectiveness of training. Only the results on right arms are shown but left arms were also tested and are described in text. Ordinates show work done in kgm. cm.

very effectively improved their ability to do work with blood supply yet no improvement in ischemic capacity occurred. The graph of subject 1 is especially instructive in this regard since he showed a steady 1.5 per cent per month improvement in ischemic ability from the start to the end. The circulated training did not seem to speed or retard this gradual process.

Subject 10. Both arms ran virtually parallel in ischemic performance, the right arm being capable of about 30 kgm. cm. more than the left. It is to be noted that the first period of circulated work apparently increased

the ischemic work capacity.   Although training continued to occur in later bouts of circulated work, the ischemic ability did not increase.

Subject 11.   This subject's right arm showed very little gain in ischemic ability as a result of circulated training.   His left arm showed no improvement whatever in ischemic ability.

Subject 5.   Returned to work after a summer vacation.   In view of his previous experience he worked his arms without blood supply for only 2 weeks before beginning circulated training.   It will be noted that his ability with 800 grams was tested from the 80th to 90th day of the graph to compare with previous year's ability as shown in table 1.   Both right and left arms showed a gain in ischemic capacity throughout the graph which was least after the last period of circulated work although training in circulated ability was greatest in the last period of circulated work.

Subject 12.   Both arms of this man ran parallel throughout the experiments with the right arm better by less than 20 kgm. cm.   In both arms the ischemic capacity showed marked improvement after the first period of circulated work but there was no further change after later periods of circulated training.

The data obtained show that the gains in ischemic work capacity obtained by daily ischemic work to fatigue for a period of months were small. Most of the trials showed no increase in ischemic work capacity as a result of effective training with blood supply.   There were several instances in which increases did occur.   Most of these instances were in the first trial of the effect of circulated training.   The question arises whether the subjects were completely acclimated to the routine of ischemic work on the ergograph before they started circulated training.   The fact that improvement in ischemic performance did not occur after later periods of circulated training suggests that the subjects were not used to the routine.

Any improvements in work capacity of muscles using the ergograph without blood supply may be due to at least two different processes: There might be an increase in skill (learning to time the movements more accurately, to execute them with less waste motion) or there might be an increase of the total energy reservoir of the ischemic muscle.   There is evidence that skill continues to improve over a fairly long period of training.   Compare the response of ischemic muscle to varying load after 4 months with the response after 14 months of daily work (subjects 1 and 2, table 2).   In the earlier trial when movements were probably wasteful and inaccurate the most work could be done with the heavier loads.   Ten months later when the movements had become more practised, the advantage of the heavy load had practically disappeared.   The comparison does reveal a small improvement in the ischemic performance with all loads as a result of the period of work.   Whether greater skill or increase in the energy reservoir is responsible is not obvious.

At all events it is clear that improvement in work capacity with blood supply does not guarantee increase in work without blood supply as is shown by a close examination of the work of the left arm of subject 2 as seen in figure 2. On the 337th and 344th days of the graph the ischemic ability of the arm was tested and found to be 540 and 525 kgm. cm. with 1100 grams' load. Due to the great reduction required for this figure these dots are unlabelled on the graph. At this time the muscle with blood supply averaged about 1350 kgm. cm. with 1400 grams' load. On the 485th and 486th days the muscle was nearing steady state ability with 1400 grams as a result of 15 minutes' daily exercise for 3 weeks with a light load. The ischemic ability was now 535 and 495 kgm. cm. On the 498th day the steady state ability with 1400 grams was well established and yet the ischemic work capacity was but 539 kgm. cm.

Training pattern in muscles working with blood supply. All subjects tested showed the same pattern seen in the graphs of the left arms of subjects 1 and 2 in figure 2. This pattern is characterized by the fact that use of light load of 800 grams gave rapid rise to steady state ability. Changing to 1100 grams fixed the capacity for work at a certain level for 150 days in subject 1, 90 days in subject 2, when both subjects showed a rapid enhancement of ability. The use of a still heavier load (1400 grams) abolished all signs of improvement in subject 1 and only very slight improvement occurred in subject 2 for 175 days. Only when an added exercise period of 15 minutes (900 contractions) of steady-state work with a lighter load was done each day did the muscles of subject 2 become able to carry 1400 grams in steady state. The added exercise did not enable subject 1 to increase his work capacity. This failure may be related to the greater age of subject 1 (45 yrs.). Yet it will be noted that the right arm of this same individual showed steady slow improvement in ischemic capacity (skill or reservoir?). Thus it would seem that if the rate of work is too fast, the muscle working in a relatively anaerobic state, training will fail to occur.

Relationship between the capacities of muscle for different types of work. The usage of muscles in the intact body may be roughly classified into three types: 1. Steady state work capacity, or endurance for aerobic work. 2. Anaerobic work capacity, ability to work without blood supply or so rapidly that the existing power to supply oxygen is negligible compared to the metabolic rate. 3. Absolute power, maximal strength exerted in a single effort. It might be supposed that a strong muscle would be strong in all these usages, a weak one poor in all of them. The data do not support this conception. The three properties seem separate. We have seen that improvement in ability to work with blood supply does not improve ability to work without it. Likewise the fact that the ischemic ability of two muscles is equal does not indicate that their circulated work

capacity will be equal. For example, the ischemic capacity of both arms of subject 2 near the end of the experiment was about 525 kgm. cm. with a load of 1100 grams. The ability with blood supply of the right was not great enough for steady state with 1100 grams whereas that of the left was sufficient to permit steady state with 1400 grams' load.

Similarly, one muscle may have poorer ischemic ability than another though the capacity of the former for training with blood supply may exceed that of the latter. Figure 3 shows that the right arm of subject 10 had an ischemic capacity of only 475 kgm. cm. but was capable of being trained to carry 1700 grams in steady state with blood supply. The right arm of subject 12 with an ischemic capacity of 700 kgm. cm. was incapable of reaching steady state with 1700 grams in the given training period.

Through failure to realize its import no attempt was made at the outset to test the absolute power of the muscles before training. The ergograph offers a convenient method of roughly estimating absolute power. Load can be increased until the unexercised muscle is just able to raise it off the ground. Making these tests near the end of the experiments revealed:

	MAXIMUM LIFT	ENDURANCE
	grams	
Subject 1 Rt...............	4350	1100 grams steady state
Subject 1 Lt...............	3750	800 grams steady state easily
		1100 grams steady state occasionally
Subject 2 Rt...............	4050	1100 grams steady state
Subject 2 Lt...............	3200	1400 grams in steady state

Thus the three rough abilities listed above seem distinct. This suggests that they may depend on different processes in the muscle.

The effect of daily ischemic work on the time of onset of ischemic pain. Katz et al. (2) concluded that training must enable the muscle to do more ischemic work before pain appears, from the fact that the right flexor was able to do more work than the left in this circumstance. This was contrary to the conclusion advanced previously by Lewis et al. (3), who considered the time of onset of ischemic pain as invariable. Table 3 shows the time of onset of pain at various periods in the course of the experiments. It is seen that the range was in most subjects quite small and the degree of change with daily ischemic practise small and slow. This would account for Lewis' conclusion that time of onset of pain was constant. In the long run most subjects showed a distinct increase in time of onset, in agreement with Katz' conclusion.

Circulated practise as well as ischemic practise tended to delay pain onset as shown by the table. The effectiveness of the various circulated

work periods in training can be compared with their effect in delaying pain by comparing numbers given for the right arm of each subject in figure 3 with the data in table 3.

A most striking effect of circulated work on ischemic pain cannot be graphically shown. As one works daily without blood supply the final intensity of the pain grows much less. If, then, one works with blood supply for a number of days the pain on returning to ischemic work is very markedly heightened. All subjects volunteered this information. No reason can be advanced to explain this phenomenon but a similar phenomenon may account for the overwhelming character of pain in intermittent claudication and angina pectoris.

TABLE 3

Effect of daily ischemic or circulated work on time of onset of ischemic pain

Each figure given is the average of 10 consecutive daily trials at the stated time

	ISCHEMIC PRACTICE										CIRCULATED PRACTICE							
		10th-20th day, Time in sec.			70th-80th day, Time in sec.			470-480th day, Time in sec.			Subject			10 days before circulated training	10 days after circulated training	After 2nd period of circulated training	After 3rd period of circulated training	After 4th period of circulated training
Subject	Load	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Number	Arm	Load					
1	800	44	51	57	64	67	72	85	89	95	1	Rt.	1100	58	65			
2	800	35	41	50	44	51	65	65	68	75	2	Rt.	1100	52	58			
3	800	45	52	60	50	55	60				5	Rt.	1100	43	43	45		
4	800	31	37	52	45	52	58				5	Lt.	1100	36	36	45		
5	800	33	36	48	40	56	60				10	Rt.	800	50	55	54	63	59
6	800	43	49	59	No pain						10	Lt.	800	49	55	55	63	
7	800	32	39	50	35	39	48				11	Rt.	1100	48	57	59	56	60
8	800	28	30	35	30	33	35				11	Lt.	1100	45	56	61	64	
9	800	43	52	60	40	64	73				12	Rt.	800	23	46	45	50	
											12	Lt.	800	23	43	65	56	

Validity of method. The literature contains many contributions which criticize and evaluate the various types of ergograph. Limitation of space prevents complete discussion of these criticisms. The most important refer to: 1. Variation of leverage as the stroke progresses results in a different amount of work per centimeter in short strokes than in long ones. This means that the meter would measure a different fraction of the work done by the muscle in a long stroke than in a short one. This objection does not apply to the present method since each stroke is of uniform length and thus the fraction of the work measured should be uniform. 2. Measuring accurately the work done with a weight ergograph is difficult due to the fact that weights have inertia. It is not claimed that the present method measures all the work done by the muscle, nor that it measures

all the work the muscle can do.   It is thought that a constant fraction
of the work done is measured, and that by working the muscle daily to a
uniform end-point of fatigue a constant fraction of all the work the muscle
can do is obtained.   The fraction of the work done which appears in our
figures must be a large one.   3. The objection can be raised that experi-
ments whose end point depends upon human judgment are apt to be in
error.   In the type of experiment reported here the subject stopped work
in ischemic exercise when he could no longer raise the load to ring the
buzzer.   How do we know he really could not raise the load?   As fatigue
sets in greater and greater effort was required to raise the load.   Asso-
ciated movements, grimaces, movements of legs, of free arm and of the
trunk increased with the effort.   The blood pressure gradually mounted.
Actual failure of power came on suddenly and could almost be predicted
by an accustomed observer.   In the work with blood supply the end point
was inability to make the full contraction in the allotted time of 1 second.
When the subject was fresh, the contractions were well spaced, he paused
in rest between them.   As he tired his efforts grew more and more con-
tinuous.   In his attempt to keep up with the rhythm he worked harder.
Associated movements were even more marked here than with the ischemic
work since the end was less sudden.   The degree of impotence of the muscle
so worked in the subject well accustomed to the routine was remarkable.
If immediately after he stopped work he was asked to raise the weight
again in a leisurely fashion it was all he could do to raise it and sound the
buzzer in 4 or 5 seconds of continuous effort.   When the subject worked
daily with the same load, it felt heavy to him on some occasions, light on
others.   Hence if without his knowledge one gave him a load different by
300 grams he could not always recognize the difference.   Thus one could
readily check on his honesty.   Such tests, watching associated move-
ments, and occasionally taking blood pressure at the end of work were
convincing evidence of the honesty of the subjects.   The actual arterial
blood pressure rise with this effort in accustomed subjects varied from 40
to 80 mm. Hg.   No tension above 200 mm. Hg was ever noted.

DISCUSSION.   The ischemic work capacity of muscle showed no sizable
increase as a result of either ischemic or circulated practise.   This sug-
gested that improvement in work capacity of circulated muscle as a result
of training must depend on changes in circulatory and central nervous
systems rather than on changes in the muscle cells themselves.   The
importance of local vascular changes in muscle training has not been
emphasized.   However, there are two logical mechanisms by which these
changes might occur.   A greater number of capillaries per muscle fiber
might be developed, and a quicker and more effective increase in flow
through existing capillaries by improvement in reflex control of vessels
would be useful.   The former mechanism is supported by the data of

Petren, Sjöstrand and Sylvèn (4) on capillary counts in gastrocnemius muscles of trained and untrained guinea pigs. They showed increases in number of capillaries per muscle fiber as great as 40 per cent above the control levels in trained animals. These changes were slower to occur and smaller in older than in younger animals.

The conception that muscle training depends on vascular changes rather than on changes within the muscle fiber is quite logical. Improvement in aerobic endurance is the principal performance change in trained muscle which has been objectively demonstrated, i.e., steady state work. The limiting factor in this kind of work is probably the ability of the blood to supply oxygen to the muscle fiber. An increase in the number of capillaries per fiber would increase the oxygen supply.

Increase in size of muscle is commonly the most emphasized effect of training. It is generally accepted that the cause of this increase in size is due to hypertrophy of the individual fiber. It is unlikely that this process would increase the aerobic endurance of muscle since it involves reduction of diffusional surface area per unit volume of muscle fiber and would thus reduce the oxygen supply. In fact, there is evidence that hypertrophy is not a uniform concomitant of muscle training. Dawson (1) states that size of muscle remains unchanged with the slow steady exercise that produces good training; only in rapid short exercise (involving sudden strain) does hypertrophy occur. The long distance runner in perfect trim shows small muscles; the weight-lifter becomes "muscle-bound."

Petren et al. (4) in their experiments on capillary counts found no difference in the size of muscle fibers from trained and control guinea pigs. Another indication that training and hypertrophy are separate processes is seen by comparing the course of the two processes. Siebert (5) showed that when rats were worked at a particular rate a certain amount of hypertrophy would occur. Persistence in work at this speed would occasion no further hypertrophy; only by increasing the speed of work could hypertrophy be continued. In contrast to this is the pattern of training reported here in muscles working with blood supply in which raising the rate of work resulted in a prolonged period of failure to gain endurance. Only when there was instituted a long exercise period with lighter load did greater endurance with the heavier load appear. Thus increase of endurance and hypertrophy are not simultaneous and may indeed be unrelated processes. There is a possibility that hypertrophy may be a by-product of training, perhaps even a noxious by-product.

Present biochemical evidence suggests that the energy release mechanisms of aerobic and anaerobic work in muscle may be separate. It is therefore reasonable that ischemic work capacity does not improve with increase in circulated work capacity.

The failure of ischemic practise to produce training, and the slow rate

of gain of endurance with rapid rates of work indicates that endurance for work with heavier loads is gained best by much practise with lighter loads.

SUMMARY

1. A weight ergograph adapted for the measurement of the work of human extensor digitorum communis muscle is described, whereby the total distance of vertical lift, the number and uniformity of individual movements made, and the stroke length are accurately gauged.

2. The muscles were voluntarily worked to fatigue once each day. The right arm was worked without blood supply, the left with blood supply. Training patterns without blood supply showed very small and slow gains in total work done amounting to an increase of, at most, 30 per cent in 16 months. The work ability of muscle working without blood supply was not significantly increased by effective training with blood supply. The work capacity of muscle without blood supply was somewhat greater with heavier than with lighter loads, but this difference was reduced by prolonged use of the machine and the development of skill. The time of onset of ischemic pain was delayed only very slowly by daily work without blood supply. The intensity of ischemic pain was markedly reduced in the process. An interim of daily work with blood supply reintensified the ischemic pain on later trials without blood supply.

3. The training pattern of muscles working with blood supply showed rapid assumption of steady state ability with light load. Shifting to a load 300 grams heavier resulted in a long period of static work capacity with finally a rapid increase in work ability. A load 300 grams heavier still abolished all signs of training for 9 months. Yet, a daily exercise period of 15 minutes with lighter load served to increase the work capacity with the heavier load.

4. It is concluded that the anaerobic energy release mechanism in muscle undergoes little change as a result of training. Training would seem to be largely a phenomenon of improvement in nervous direction and vascular supply.

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OVULATION INDUCED IN MICE BY SINGLE INJECTIONS OF FOLLUTEIN[1] OR UNTREATED HUMAN PREGNANCY URINE[2]

H. O. BURDICK AND RAE WHITNEY

From the Department of Biology, Alfred University, Alfred, New York

Since Friedman (1) showed that ovulation could be induced in the rabbit by a single intravenous injection of the urine of pregnant women, numerous investigators have experimented with other animals to determine whether they will similarly respond to gonadotropins in pregnancy urine (PU).

In mice, ovulation induced by PU substances has been reported, but this seems to occur only after the animals have been injected for several days. This is particularly true of the immature mice. Hill (2) observed ovulations in 21-day old mice after four daily injections and in 14-day old animals treated for six days. Nelson and Overholser (3) reported ovulations in mice 20 to 22 days old, after injecting Antuitrin-S or pregnant mare serum twice daily for three days. Osborn (4) induced ovulation in dwarf mice, which are usually sterile, after first stimulating the ovaries with FSH twice daily for four days, then injecting LH.

Since these other investigators were concerned with the results of several injections, this experiment was undertaken to determine the possibility of inducing ovulation in mature, non-pregnant mice, pregnant mice and late immatures by a single subcutaneous injection of either the commercial extract, Follutein, or untreated human pregnancy urine.

PROCEDURE. The albino mice used in these experiments were from the Rockland strain. Each animal received only one subcutaneous injection. At first only mature unmated females were injected with Follutein. Later, when it was found that Follutein regularly induced ovulation, a smaller number of mature unmated, mated, and also immature female mice were similarly injected with 0.5 cc. of untreated urine from human pregnancies of approximately two and one-half months' duration. This amount of urine, chosen empirically, gave consistent results, and was easily tolerated. These animals were autopsied 18 to 20 hours after the injection. No attempt was made to find minimal ovulation doses.

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[1] We are indebted to Doctors J. A. Morrell and G. A. Harrop of E. R. Squibb and Sons for a generous supply of Follutein.

[2] The human pregnancy urine was obtained through the coöperation of Dr. R. O. Hitchcock, Alfred, New York, and Dr. H. S. Brasted, Hornell, New York.

Each half of the reproductive tract was removed intact, washed with normal saline solution, and placed in a deep-well slide for inspection under a dissecting microscope. The ampulla of the oviduct was then carefully examined by reflected light. Ovulation was plainly indicated by the appearance of the ampulla, which was distended by fluid and granulosa cells accompanying the eggs. The extent of ampullar distention was, of course, proportional to the number of eggs and the amount of fluid present.

Fig. 1. Photomicrograph of a mouse ovary, *O*, uterus, *U*, and fallopian tube, *T*, as they appear under the microscope before either removal of the ovary or straightening of the tightly coiled oviduct. Arrow points to ova and granulosa cells within the distended ampulla. The photomicrograph, × 12, was taken by means of reflected light.

Fig. 2. Photomicrograph of the ampulla of a mouse oviduct. Flattening the distended ampulla by pressure on the cover glass has separated the cumulus cell mass into component ova with cumulus cells. Eight ova can be seen within the thin ampullar walls. Photomicrograph taken with transmitted light.

Two methods have been used for determining the contents of an ampulla. First, when it was desired to determine only the presence and not the number of ova, the tube was exposed to the light of a good microscope lamp directed down onto the stage at about a 45° angle while the ovary and folds of the mesosalpinx were manipulated with fine-pointed Boley forceps. In this light, a mass of granulosa cells could be distinguished within the thinned ampullar wall (fig. 1) and, usually, individual eggs could be seen as grayish-white spheres surrounded by halos of cumulus cells.

The second method, hereinafter referred to as the pressure method, took a little more time but was the better procedure for counting the eggs. The tract in a deep-well slide under the dissecting microscope was so exposed that the ovary could be cut away from the oviduct. Then the coiled oviduct was separated from the uterus, together with a small portion of that organ which was used for handling the oviduct during later operations. The mesosalpinx, which normally holds the ovary close to the anterior end of the uterus and helps to make a coil of the oviduct, was severed in one or two places to allow a partial straightening of this tube. For the observation of the tract during this procedure, a substage light is

TABLE 1

Ovulation induced with follutein, in mature non-pregnant mice

ANIMAL NUMBER	AUTOPSY HOURS AFTER INJECTION	TUBAL OVA WITH GRANULOSA CELLS	ANIMAL NUMBER	AUTOPSY HOURS AFTER INJECTION	TUBAL OVA WITH GRANULOSA CELLS
Injection: 300 rat units			Injection: 200 rat units		
1	24	7	1	29	9
2	24	9	2	28	6
3	23	6	3	28	3
4	23	10	4	27	No ovulation
5	20	8	5	24	12
6	16	5	6	20	3
			7	18	8
			8	14	4
			9	13	No ovulation
			10	12	No ovulation
			11	11	No ovulation
			12	11	No ovulation
			13	10	8
			14	9	No ovulation

sometimes superior to reflected light. However, individual eggs in the ampulla could always be seen much more clearly if a microscope cover glass were lowered onto the partially straightened oviduct. Gentle pressure applied to the cover glass flattened and squeezed aside the granulosa cells sufficiently to show each egg. Care was taken not to rupture the swollen ampulla. Figure 2 is a photomicrograph of a typically distended ampulla which was flattened by this pressure method. The egg cluster has been broken up so that at least 8 ova with halos of cumulus cells can be counted.

RESULTS. A. *Effect of Follutein on normal, non-pregnant mice.* Mature mice were injected once only with 200 or 300 R.U. of Follutein, and autopsies were performed 9 to 29 hours later. An examination of table 1 shows that an injection of 200 R.U. is apparently as effective in causing ovulation as is the higher dosage. Fourteen (70 per cent) of the 20 animals

had ovulated, and of those autopsied 14 or more hours after the injection, all but 1 (approximately 93 per cent) had eggs with granulosa cells in the ampulla. Of the six autopsied earlier than 14 hours after the injection, only 1 had ovulated. This was mouse 25, with eggs 10 hours after the injection, but this animal may have been in estrus and about to ovulate spontaneously.

TABLE 2

Induced ovulation with 0.5 cc. untreated human pregnancy urine

ANIMAL NUMBER	TUBAL OVA		TOTAL	COMMENTS
	Right	Left		
Mature unmated female mice				
1	3	6	9	
2	5	5	10	
3	9	6	15	
4	5	13	18	
5	4	4	8	
6	5	7	12	
7	5	5	10	
8	6	2	8	
Mated female mice				
1	0	0	0	1-celled ova in oviducts without granulosa cells
2	0	0	0	4-celled ova in lower oviduct
3	0	0	0	2-celled ova in oviduct
4	6	12	18	4 implants about 18th day of pregnancy
5	2	8	10	8 small implants 7 to 8 days
6	8	4	12	6 small implants 7 to 8 days
7	9	7	16	12 implants 12 to 13 days
Immature female mice: Vaginal orifice closed				
1	0	0	0	11.5 grams
2	0	0	0	12.0 grams
3	0	0	0	12.8 grams
4	1	6	7	14.0 grams
5	2	2	4	14.4 grams
6	13	8	21	15.0 grams

These results are particularly interesting because they show that ovulation can be expected 10 to 15 hours after the PU extract injection.

B. *Results following a single injection of 0.5 cc. pregnancy urine.* 1. *With mature non-pregnant female mice.* Ovulation was induced in each of the 7 animals of this series. The total number of ova in both ampullae varied from 8 to 18 with an average of 12 eggs, which is more than the usual number in a normal ovulation. (See table 2.)

2. *With pregnant female mice.* The results in this group are of interest for two reasons. First, in none of the 3 animals with early embryos still unimplanted, had induced ovulation taken place. No conclusions could be drawn from so small a number of animals, but it is possible that there is a definite refractory period following normal ovulation, or else that the dosage is not correct to induce ovulation under these conditions.

In the second place, however, ovulation was readily induced after implantation of embryos had taken place. Animals with small beaded uteri (7 to 8 days *post coitum*) responded to the injections with 10 to 12 eggs each. One animal in late pregnancy (16 to 18 days) was found with a total of 18 eggs in the ampullae. These results show that these ovulations were not cyclic but were induced by the injected pregnancy urine.

3. *With late immature female mice with unopened vaginal orifices.* It must be admitted that this phase of the experiment was undertaken with little expectation of positive results. However, the first animal autopsied (weight 14 grams, approximately 35 days old) had 7 eggs in the ampullae. The animals weighing 14 grams or more ovulated, but none of those weighing less than 13 grams had ovulated. (See table 2.)

Although all of the animals in this series were late immatures, in no instance had the vaginal orifice opened at the time of autopsy.

DISCUSSION. One may now wonder whether some of the earlier experiments of others on the luteinization of mouse and rat ovaries might not have been accompanied by unobserved, induced ovulations. This could have occurred when the animals were injected for several days, and when the autopsies were delayed until after the eggs had had time to disintegrate. On the other hand, some of the injections of other investigators were quite likely to have been excessive dosages that resulted in increased luteinization which, in turn, inhibited ovulation. However, sub-threshold amounts injected for several days are also known to cause luteinization without ovulation. Friedman (1) obtained such results in the rabbit with sub-minimal doses of prolan. These differences in results serve to emphasize the importance of time-dosage factors.

The experiments reported here also show that ovulations probably occur earlier than suspected. During the first 24 hours after the supposed time of ovulation, the ampulla should be carefully examined for eggs surrounded by granulosa cells, but later than this the eggs may be denuded of these sticky cells and found in lower regions of the tubes or in the uteri. In fixing the time of induced ovulation in these mice at 10 to 15 hours after a single subcutaneous injection, we find that these results are comparable to those of Friedman (1), who found ovulation in rabbits 10 to 15 hours after one intravenous injection of PU. Since ovulation-time after subcutaneous injections could be expected to be somewhat longer than by

the intravenous route, we are at present autopsying our mice 16 to 18 hours after injections.

At first it might appear that normal female mice selected at random were not suitable animals for this experiment, since their estrous cycles are usually only 4 to 5 days, and some of the mice, selected by random, might be expected to ovulate within 15 hours after the injection. However, the induction of ovulation in pregnant and immature mice indicates that the ovulations following PU extracts in mature unmated animals are not, to any great extent, the result of fortuitous timing of the injections.

SUMMARY

Single subcutaneous injections of the urine extract, Follutein (Squibb), induce ovulation within 15 hours in mature, non-pregnant female mice.

Similar injections of 0.5 cc. of untreated human pregnancy urine likewise induce ovulation in mature, non-pregnant female mice, and also in pregnant animals with implanted embryos, and in late immature mice with unopened vaginal orifices.

Animals in early pregnancy, with fertilized ova still in the oviducts, appeared to be refractory to this amount of pregnancy urine, and immature females weighing less than 13 grams also failed to ovulate.

A method for counting the ova within the oviduct is presented, and the general appearance of the ampulla after an induced ovulation is shown in photomicrographs.

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RELATIONSHIP OF THE PLASMA VOLUME AND THE CELL PLASMA RATIO TO THE TOTAL RED CELL VOLUME

EUGENE A. STEAD, JR. AND RICHARD V. EBERT

From the Medical Clinic, Peter Bent Brigham Hospital, Department of Medicine, Harvard Medical School, Boston, Mass.

The proportion of cells and plasma in blood removed from the body is determined by the hematocrit. As the hematocrit reading of blood taken from the arteries, capillaries and veins of the extremities is approximately the same, and as in dogs blood from the viscera with the exception of the spleen has the same hematocrit reading as blood from the extremities (1, 2), many investigators have assumed that by the use of the hematocrit the actual cell plasma ratio of the circulating blood can be obtained. On the basis of this assumption, many workers have calculated the red cell volume from the plasma volume and hematocrit reading and have estimated changes in plasma volume from changes in the cell plasma ratio or hemoglobin concentration. Smith, Arnold and Whipple (2), however, believed that the hematocrit reading did not give the true cell plasma ratio of the blood as a whole. They demonstrated that the red cell volume as determined by the CO method and the Welker method was lower than the red cell volume determined from the plasma volume (dye method) and the hematocrit reading. Since the CO and Welker methods for measuring the red cell volume did not depend on the hematocrit reading, they concluded that these methods gave the correct red cell volume, and that the red cell volume calculated from the plasma volume and hematocrit reading was falsely high because the hematocrit did not give the correct cell plasma ratio of the entire blood. It was further shown (3) that when the hematocrit reading was lowered by bleeding, the measured red cell volume (from the plasma volume and hematocrit reading) did not agree with the red cell volume predicted on the basis of the volume of red cells removed.

In the study of the circulation, it is essential to know whether the hematocrit reading represents the true cell plasma ratio of the circulating blood. If it does not, in what direction is the error and what is its magnitude? With an accurate method for measuring the plasma volume, this information can be obtained by determining the plasma volume and hematocrit reading before and after removal of a known quantity of red cells. The

red cell volume is calculated from the plasma volume and hematocrit reading. If the red cell volume before hemorrhage is equal to the red cell volume after hemorrhage, plus the volume of the red cells removed, the hematocrit gives the correct cell plasma ratio of the entire blood; if the red cell volume before hemorrhage is greater than the red cell volume after hemorrhage plus the volume of the red cells removed, the hematocrit reading does not represent the cell plasma ratio throughout all of the blood. In normal human subjects the red cell volume 72 hours after venesection always appeared lower than the red cell volume predicted from the pre-hemorrhage red cell volume and the volume of red cells removed (4). The changes in hematocrit readings were, however, relatively small. This paper reports the results of similar experiments in dogs in which a marked drop in hematocrit reading was produced by massive bleeding.

METHOD. Six complete experiments were performed on 4 unanesthetized, splenectomized dogs. The spleens were removed because it has been shown that in dogs under certain conditions the spleen discharges blood rich in cells into the general circulation (5). The circulation in splenectomized dogs resembles that of normal human subjects because in man there are no significant blood reservoirs (6). The plasma volume, hematocrit reading, and hemoglobin concentration were determined on two successive days. The red cell volume was calculated from the plasma volume and hematocrit reading, the total circulating hemoglobin from the total blood volume and the hemoglobin concentration in grams per cent. If the values for these determinations checked within 5 per cent on two successive days, bleeding was begun in the next 24 hours. A 2 per cent solution of sodium citrate was used as an anticoagulant. The hematocrit reading and hemoglobin concentration of the blood and citrate mixture were determined, and the total volume of red cells and the total grams of hemoglobin removed were calculated. At the end of each bleeding the plasma of the blood removed was separated from the red cells and returned to the dog. Within 48 hours from the time of the first bleeding the plasma volume, the hematocrit reading, and hemoglobin concentration were again determined.

The plasma volume was measured by the dye method of Gregersen, Gibson and Stead (7) as adapted to the Evelyn micro-colorimeter (8). The hematocrit was determined by the method previously described, using 1.6 per cent solution of potassium oxalate as the anticoagulant (9). The total blood volume was calculated from the plasma volume and hematocrit reading. The hemoglobin concentration in grams per 100 cc. of blood was determined by the method of Evelyn (10). The jugular vein was used for injecting the dye and for taking blood samples. Stasis was carefully avoided.

RESULTS. The results were essentially the same in all 6 experiments (table 1). The prehemorrhage red cell volume (calculated from the

plasma volume and hematocrit reading) was from 21 to 34 per cent greater than the red blood cell volume after hemorrhage (calculated from the plasma volume and hematocrit reading) plus the volume of red cells removed by bleeding. Obviously, if the red cell volume calculated from the plasma volume and hematocrit reading was an accurate measurement, these two values would have been identical. The estimation of the prehemorrhage red cell volume from the plasma volume and hematocrit reading is not as accurate as is the calculation of the prehemorrhage red cell volume by adding the volume of red cells removed by venesection to the red cell volume after hemorrhage. In the former the entire measurement is an indirect one based on the assumption that the hematocrit actually

TABLE 1

Typical experiment demonstrating error in calculation of red cell volume from plasma volume and hematocrit reading

DATE	PLASMA VOLUME	RED CELL VOLUME	HEMATOCRIT READING	HEMOGLOBIN	TOTAL CIRCULATING HEMOGLOBIN	NUMBER OF TIMES BLED	RED CELLS REMOVED	HEMOGLOBIN REMOVED	VOLUME OF RED CELLS REMOVED PLUS RED CELL VOLUME AFTER BLEEDING	ESTIMATED PER CENT ERROR IN PREHEMORRHAGE CELL VOLUME	HEMOGLOBIN REMOVED PLUS TOTAL CIRCULATING HEMOGLOBIN AFTER BLEEDING	ESTIMATED PER CENT ERROR IN PREHEMORRHAGE TOTAL CIRCULATING HEMOGLOBIN
	cc.	cc.		grams/100 cc.	grams		cc.	grams	cc.		grams	
7-10-40	1,140	1,390	55.0	18.3	463		25	8				
7-11-40						3	784	274				
7-12-40	1,610	230	12.7	4.2	77				1,039	34	359	29
8-27-40*	1,280	1,180	48.0	15.4	379		25	8				
8-28-40						3	702	233				
8-29-40	1,610	200	10.9	3.4	62				927	27	303	25

* The dog was not bled from 7-12-40 to 8-27-40.

determines the cell plasma ratio of all the blood in the body; in the latter 75 per cent of the value for the red cell volume is determined by accurate measurements outside the body, and only 25 per cent is dependent upon the indirect measurement involving the assumption that the hematocrit determined the cell plasma ratio of all the blood in the body. The true red cell volume before hemorrhage is, therefore, approximated by adding to the volume of the red cells removed by venesection the red cell volume after venesection (from the plasma volume and hematocrit reading). The value obtained for the prehemorrhage red cell volume as determined from the hematocrit reading and plasma volume is falsely high. The total circulating hemoglobin as estimated from the total blood volume (from plasma volume and hematocrit reading) is also falsely high (table 1). The falsely high red cell volume as determined from the plasma volume

and hematocrit reading can be explained in three ways: 1, the plasma volume technique gives a falsely high plasma volume; 2, the hematocrit does not determine the true cell plasma ratio of the blood removed, because plasma is trapped among the packed red cells; 3, the cell plasma ratio of the circulating blood as a whole is lower than the hematocrit reading of blood drawn from the body.

If dye were lost into the tissue fluids and returned into the blood stream during the time of the determination, the value for the plasma volume would be too high. When the plasma volume is calculated from the slope of the disappearance curve of the dye, loss of dye into the extracellular fluids would not affect the determination unless large quantities of dye-stained lymph enter the blood stream during the determination. The lymph flow from the thoracic duct of fasted resting dogs (11) is so slow that the entry of dye-stained lymph could not cause an appreciable error in the plasma volume technique.

By calculating indirectly the cell plasma ratio of blood drawn into a flask, it was possible to demonstrate that the hematocrit reading, as determined in the routine manner, represents the true cell plasma ratio of the blood removed from the body and that an appreciable amount of plasma is not trapped among the red cells. Approximately 100 cc. of blood were withdrawn from the antecubital vein of a normal subject in a syringe coated with vaseline. The blood was placed in a paraffin flask containing 0.2 cc. of 0.1 per cent solution of Evans blue and the flask was rotated for 5 minutes. At intervals blood was placed in sample tubes and allowed to clot. The total quantity of blood was determined from the weight of the blood removed and the specific gravity. The cell plasma ratio was determined *a*, from the hematocrit reading in the usual manner, using 1.6 per cent solution of potassium oxalate as the anticoagulant, and *b*, from the plasma volume as calculated from the dye concentration and the total quantity of blood. The dye concentration in the serum of the samples of clotted blood was measured by the microcolorimeter in the usual manner. Two experiments were performed using blood drawn from two different subjects. In each case the cell plasma ratio, as calculated by the dye method, agreed within 3 per cent with that determined by the hematocrit (table 2). Using a slightly different technique, Gregersen and Schiro obtained similar results (12).

The third explanation is, therefore, the correct one. The cell plasma ratio of blood circulating in the entire body is lower than that of blood removed from the body. The red cell volume after hemorrhage plus the volume of red cells removed is, therefore, less than the red cell volume determined before hemorrhage, because the hematocrit reading does not measure the cell plasma ratio of the entire blood. From these experiments it appears that when the hematocrit reading is between 40 and 50 the red

cell volume as measured from the plasma volume and hematocrit reading is about 25 per cent higher than the true red cell volume. In calculating the total volume with a hematocrit reading of 50 this error is reduced by about one-half, making the measured total volume about 11 per cent higher than the true total volume.

When the hematocrit reading is between 40 and 50, the total circulating hemoglobin as measured from the total blood volume and the concentration of hemoglobin in grams per cent is approximately 25 per cent higher than the true total circulating hemoglobin. Both of the values used in the calculation, namely, the total blood volume and the hemoglobin concentration, are falsely high. The fact that the error in total circulating hemoglobin parallels the error in the total red cell volume indicates that changes in the size of the individual cells played no rôle in these experiments.

TABLE 2

Calculation of the volume of cells in blood drawn into a flask from the volume of blood and the volume of plasma

A known amount of dye was added to the blood and the plasma volume was calculated from the concentration of dye in the serum.

SUBJECT	VOLUME OF BLOOD	VOLUME OF PLASMA AS CALCULATED FROM THE CONCENTRATION OF DYE	PREDICTED HEMATOCRIT READING FROM VOLUME OF BLOOD AND VOLUME OF PLASMA	HEMATOCRIT READING
	cc.	*cc.*		
E. S.	93	52.5	43.5	45.0
R. E.	100	54.5	45.5	46.3

DISCUSSION. These observations support the conclusions of Smith, Arnold and Whipple (2) that the red cell volume cannot be measured from the plasma volume and hematocrit reading. By a comparison with the CO and Welker methods, they concluded that with a hematocrit reading of 50 the value obtained for the red cell volume from the plasma volume and hematocrit reading was approximately 20 per cent higher than the actual red cell volume. Although a different method was used in this study, than that used by Smith, Arnold and Whipple, the results agree fairly closely as to the magnitude of the error when the red cell volume is calculated from the plasma volume and hematocrit reading.

The physical characteristics of blood flowing in small tubes satisfactorily explain the apparently paradoxical phenomenon that although the blood removed from artery, capillary and vein has the same cell plasma ratio, this is not the cell plasma ratio of the total circulating blood. Fåhraeus (13) compared the hematocrit reading of blood flowing through a fine tube (obtained by sealing the ends of the capillary tube and centrifuging

it) with the hematocrit reading of the blood that had flowed out of the tube. The cell plasma ratio of the blood dripping from the tube was higher than that of the blood within the tube. He concluded that blood streaming through narrow tubes is relatively much richer in plasma and poorer in corpuscles than the same blood streaming through larger tubes. He points out that it is impossible to obtain the hematocrit reading of blood flowing in a narrow vessel by collecting blood from the cut end of the vessel. The axial portion of the stream, which is rich in red cells, flows out of the cut end while the slower moving peripheral layer of plasma remains in the vessel.

SUMMARY AND CONCLUSIONS

1. Six bleeding experiments were performed on four splenectomized, unanesthetized dogs. The plasma volume and hematocrit reading were determined before hemorrhage. After the removal of a known quantity of red cells, the plasma volume and hematocrit reading were again determined. The red cell volume before hemorrhage was calculated *a*, from the prehemorrhage plasma volume and hematocrit reading, and *b*, from the sum of the post-hemorrhage red cell volume (plasma volume and hematocrit reading) and the volume of red cells removed. Evidence is presented to show that the calculation of the red cell volume from the sum of the post-hemorrhage red cell volume and volume of red cells removed represents approximately the true red cell volume.

2. When the hematocrit reading is approximately 50, the red cell volume as calculated from the plasma volume and hematocrit reading is approximately 25 per cent higher than the true red cell volume. This error occurs because the cell plasma ratio of blood removed from the body is higher than that of the entire circulating blood.

3. The hemoglobin concentration of blood removed from the body is higher than the hemoglobin concentration of the entire circulating blood. When the hematocrit reading is approximately 50, the total circulating hemoglobin is approximately 25 per cent higher than the true total circulating hemoglobin.

4. As the hemoglobin concentration and the cell plasma ratio of blood removed from the body are not representative of all the blood in the vascular system, it is not possible to accurately quantitate changes in plasma volume from changes in either the hemoglobin concentration or hematocrit reading.

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DISTRIBUTION OF WATER AND ELECTROLYTES IN EXPERIMENTAL DIABETES MELLITUS[1]

F. WILLIAM SUNDERMAN AND F. C. DOHAN

From the Department of Research Medicine, The William Pepper Laboratory of Clinical Medicine and the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia

Although the composition of the serum in experimental diabetes mellitus and in patients suffering with the disease has been studied at length, comparatively few data are available regarding the total quantities of the serum components or of the distribution of body water present in the various stages of this disease. Quantitative chemical analyses are limited to estimations of concentration. To determine the total amount of a component in the serum the additional measurement of serum volume is obviously necessary.

The few measurements of serum volume reported in cases of diabetes mellitus suggest that in the milder group the serum volume is not altered in any special manner but perhaps is subjected to greater variations than is observed in normal individuals. In the more severe group, and especially in individuals with ketosis, the measurement of serum volume by the more recent dye methods is practically precluded because of the undesirability of withholding treatment during the length of time required for making the measurement (i.e., $1\frac{1}{2}$ to 2 hrs.). Chang, Harrop and Schaub (1) employing the carbon monoxide method for measuring blood volume found that the total plasma volumes may be diminished as much as 24 per cent when compared to the values obtained after recovery.

Under certain conditions relative changes in the amounts of given components circulating in the serum may be estimated without the measurement of serum volume. If it be assumed that during a period of observation the amounts of serum protein or serum solids remain unchanged and that differences in their respective concentrations are due to loss or gain of serum water, then the changes in the quantities of serum components may be calculated from these differences. Utilizing this indirect method we have reported from this laboratory (2) (3) changes in the quantities of various serum constituents following the ingestion of glucose in diabetic individuals and following the administration of large

doses of insulin to severe diabetic patients. Assuming a constancy in the amounts of protein in the serum, the studies of Peters and his associates (4) (5) indicate that during diabetic ketosis the deficit in chloride and total base is greater than the diminished concentrations of these components would indicate.

The present study was designed to determine, by direct methods, the changes in content of components in the serum of dogs during various stages of experimental diabetes mellitus induced by pancreatectomy.

PROCEDURE. Large, healthy dogs were acclimatized in their cages for a week or more before studies were made. After this preliminary period and a fasting period of 24 hours, the animals were given nembutal parenterally, following which measurement of serum volume was made and blood was obtained for chemical analyses. Total pancreatectomy was then performed. After operation the animals were given maintenance dosages of insulin until recovery from the operation had occurred.

After the animals were in good condition, insulin therapy was completely withdrawn and the animals were permitted to go into ketosis. Final measurements were made when ketonuria was well developed but before circulation became impaired. The period of time required for the development of severe ketosis varied greatly with individual dogs, some remaining active for as long as 3 weeks following withdrawal of insulin. All of the animals had heavy ketonuria for several days before the final measurements were made, although they did not all yield greatly diminished concentrations of CO_2 in the serum. Reliance could not be placed entirely upon the level of CO_2 concentration in the serum in establishing the condition of an animal. Two animals died before the final observations could be made and in one of these the CO_2 concentration was 35 volumes per cent shortly before death.

Since all of the dogs suffered severe reductions in body weight, the effect of simple starvation was studied in 2 animals. Measurements of serum volume and serum electrolytes were made in these dogs before and after losing approximately 25 per cent of their body weights.

The measurement of serum volume was made with vital red using the Sunderman and Austin method (6). Measurement of total base was made by means of the procedure recommended by Hald (7). Estimation of the extracellular water was made by injection of NaCNS following the method of Crandell and Anderson (8). Fatty acids were analyzed by the Mann and Gildea (9) method. Methods for the other analyses have been given in previous publications from this laboratory (10).

In 2 animals portions of rectus muscle (2 to 3 grams net weight) were removed before pancreatectomy and before ketosis. The amounts of solids in these sections were obtained by drying them to constant weight at 105°C.

RESULTS. The results of the serum analyses are given in table 1. It will be observed that from the time of the initial measurements, when the dogs were in a normal healthy state, until the final measurement there was a loss of body weight amounting to from 25 to 43 per cent.

The initial measurements of serum volume expressed in relation to body weight were all practically within the same range of values obtained previously on other normal dogs in this laboratory (45 to 55 ml. per kgm. of body weight). During ketosis the total amounts of serum were decreased in all of the dogs. The serum volumes in relation to body weights were however all increased. Although no initial measurement was obtained in dog 2, nevertheless the determination made during ketosis in this animal yielded a serum volume in relation to body weight that was considerably above the upper normal level. The serum volumes per kilogram of body weight following starvation in dogs 7 and 8 were practically the same as initially,—the total amounts of serum obviously being diminished.

The concentrations of serum *sugar* were increased in all of the depancreatized animals during ketosis and the CO_2 concentrations decreased. The concentrations of *urea nitrogen* were measured in 4 animals and found to be increased during ketosis in 3 of them. In all of the dogs excepting dog 6 the concentrations of serum *solids* and *protein* were decreased in ketosis,—the water per kilo of serum being increased. In table 1 are given the amounts of water per kilo of serum determined from specific gravity data using the conversion formula of Sunderman (11). Although the original factors were obtained from measurements on human sera it will be seen that when applied to dog sera the calculations derived from the formula agree satisfactorily with the values obtained by analysis.

The concentrations of serum *fatty acids* were increased during ketosis in 3 animals in which they were measured. The concentrations of *total base*, *chloride*, and CO_2 were decreased in the sera of all of the depancreatized dogs during ketosis. In the sera of 3 of the experimental animals, measurements of the freezing point were made. During ketosis the *freezing point depressions* of the sera were increased above those obtained initially. The decrease in osmolar concentrations of total base during ketosis was more than compensated by the increased osmolar concentrations of serum sugar and other non-electrolytes.

The concentration of undetermined anions was obtained by adding together the values of BCl, $BHCO_3$ and BPr[2] and subtracting this sum from the total base value. In all of the dogs excepting dog 2 the concentrations of undertermined anions were increased during ketosis.

Since all of the diabetic dogs suffered severe reductions in body weight

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[2] $BHCO_3 = [CO_2] - 1.27$
$BPr = 0.97(Pr)(pH - 5.26)$

TABLE 1

Concentration of serum components

DOG	DATE		BODY WEIGHT (kgm.)	SERUM VOLUME (mL.)	SERUM VOLUME (ml./kgm. B.W.)	SUGAR (mg/100 mL.)	UREA N (mg/100 mL.)	SOLIDS (gm/100 gm.)	PROTEIN (gm/100 mL.)	SP. GR. (20°/20°)	WATER By analysis (gm./kgm.)	WATER From sp. gr. (gm./kgm.)	FATTY ACIDS (mM/l.)	CHOLESTEROL (mg/100 mL.)	TOTAL BASE (m.eq./l.)	TOTAL CHLORIDE (m.eq./l.)	CO_2 (vol. per cent)	FREEZING POINT (°C.)	UNDETERMINED ANION (m.eq./l.)
1	9/28	Control before pancreatectomy	11.1	628	56.9	88	24	7.99	6.0	1.0245	920	921	7.8	184	149.6	107.5	58	−0.567	5.9
	10/20	Ketosis after pancreatectomy	7.5	606	80.6	440	30	6.21	4.8	1.0210	938	932	12.4	167	140.0	99.8	49	−0.597	10.3
2	10/22	Control before pancreatectomy	16.2	737	80.1	117	27	7.23	5.2	1.0223	928	927	9.7	258	145.2	107.6	57	−0.565	3.3
	11/21	Ketosis after pancreatectomy	9.2			520	18	6.27	4.8	1.0209	937	932	20.3	208	135.0	105.8	41	−0.581	2.9
3	1/19	Control before pancreatectomy	18.4	974	52.9	110	19	7.21	5.2	1.0222	928	927	10.3	200	146.1	109.8	55	−0.562	3.0
	2/15	Ketosis after pancreatectomy	12.7	873	68.6	430	42	6.43	4.8	1.0210	936	932	18.2	200	144.4	100.3	34	−0.600	20.9
4	6/23	Control before pancreatectomy	19.7	918	46.6	116	20		4.9						149.1	111.1	58		3.8
	8/6	Ketosis after pancreatectomy	12.6	669	53.3	460	29		4.5						137.9	99.4	31		17.2
5	11/29	Control before pancreatectomy	15.8	873	55.3	104		6.79	5.0	1.0217	932	930		125	149.5	112.4	59		2.4
	12/16	Ketosis after pancreatectomy	11.3	818	72.7	440		6.40	4.5	1.0201	936	936		176	136.6	98.5	39		13.3
6	12/17	Control before pancreatectomy	33.8	1780	52.5	100		7.81	5.9	1.0243	922	922		143	147.1	107.6	56		4.2
	1/4	Ketosis after pancreatectomy	25.5	1696	66.7	412		8.36	6.7	1.0265	916	914		169	144.6	105.2	27		15.6
Average values		Controls before pancreatectomy			52.8	106	22	7.44	5.4	1.0230	926	925	9.3	182	147.8	109.4	57	−0.565	3.8
		Ketoses after pancreatectomy			70.3	450	30	6.72	5.0	1.0219	933	929	17.0	184	139.8	101.5	37	−0.593	13.4
7	3/30	Before starvation	16.2	864	53.3	111		8.02	6.3	1.0253	920	918		183	151.6	110.3	55		5.9
	4/18	After starvation	12.1	655	54.4	102		7.42	5.8	1.0239	926	923		151	149.2	106.6	47		11.6
8	1/4	Before starvation	18.6	956	51.4	127		8.20	6.3	1.0253	918	918		104	152.0	106.5	64		6.2
	1/29	After starvation	13.6	654	48.1	119		7.58	5.8	1.0238	924	924		131	156.5	113.3	58		7.5
Average values		Before starvation			52.4	119		8.1	6.3	1.0253	918	918		144	151.9	108.4	60		6.1
		After starvation			51.3	111		7.5	5.8	1.0239	925	924		141	152.9	110.0	53		9.6

comparisons of similar measurements were made in dogs subjected to simple starvation. The results of these measurements are given in table 1. With the exception of reduction in concentrations of serum protein the values for other serum components exhibited no essential change during starvation.

The amounts of components in the circulating serum were calculated during the control period and during ketosis and have been compared in two ways. First, the total amount of a given component in the serum during ketosis was compared to the total amount of that component present during the control measurements. Second, the total amount of a given component per kilogram of body weight during ketosis was compared to the total amount of that component per kilogram of body weight during the control measurements. In table 2 is given the average of the percentile

TABLE 2

Average of percentile changes in serum contents during ketosis

	WEIGHT	SERUM VOLUME	WATER	TOTAL BASE	Cl	BPr	BHCO₃	SUGAR	CHOLESTEROL
Diabetes									
Total change........	−33	−10	−0.8	−15	−16	−16	−45	+282	+6
Change per unit of body weight.......		+29	+33	+23	+25	+20	−17	+431	+49
Starvation									
Total change........	−26	−28	−31	−30	−28	−20	−38	−31	−14
Change per unit of body weight.......		−2	−2	−2	−1	−13	−7	−7	−16

changes in the serum contents during ketosis as related *a*, to the total amount present in the circulation during the control period, and *b*, as related to the amount present per kilogram of body weight during the control period. The values in the depancreatized animals are compared with those obtained in the starved animals.

From the time of the initial to the final measurements the diabetic dogs lost weight amounting to from 25 to 43 per cent with an average loss of 33 per cent. Final measurements were made in the starved animals after loss of 26 per cent of the body weight.

It will be seen that although the actual amount of serum present in the circulation during diabetic ketosis was 10 per cent less than that in the healthy state, the serum volume per kilogram of body weight was on the average 29 per cent greater than that in the healthy state. In the starved animals the total serum volume was decreased in proportion to the reduction in weight and within the limits of error of our measurements the serum volume per kilogram of body weight was unchanged.

In the diabetic dogs the total amount of water in the serum during ketosis was unchanged although the serum water per unit of body weight was increased 33 per cent. In the starved dogs the total serum water was decreased in proportion to the decrease in body weight, so that the serum water per unit of body weight was essentially unchanged. The actual amounts of total base, Cl, and BPr were all decreased during ketosis, although the amounts of these components per unit of body weight were all increased. In the starved animals the total amounts of these components, excepting BPr, exhibited essentially no change during ketosis when expressed in relation to the body weight.

TABLE 3

Extracellular water in dogs

DOG		BODY WEIGHT	EXTRACELLULAR WATER		SOLIDS RECTUS MUSCLE
		kgm.	*kgm.*	*per cent of body weight*	*gm./100 grams wet tissue*
5	Control	15.3	4.89	31.0	24.8
	Diabetic ketosis	11.3	4.89	43.5	23.8
6	Control	33.8	10.64	31.5	26.6
	Diabetic ketosis	25.5	10.16	39.9	26.1
7	Control	16.2	5.44	33.6	
	Starvation	12.1	4.51	37.3	
9	Control	28.4	8.59	30.2	
10	Control	27.1	7.87	29.1	
11	Control	21.6	6.26	29.0	

From the data in table 2 it will be seen that of all the serum components studied in the diabetic dog and expressed in relation to body weight, the bicarbonate content alone per kilogram of body weight was consistently decreased. These analyses of the serum in the depancreatized animals are in marked contrast to those obtained during the ketosis of starvation. In the starved animals the amounts of the serum components as well as the serum volume per unit of body weight remained practically unchanged with the exception of protein and cholesterol which were significantly decreased.

Extracellular water. The results of the measurements of extracellular water in dogs 5, 6 and 7 are given in table 3. The concentrations of thiocyanate were calculated in relation to the serum water employing the conversion factors obtained from specific gravity data (11). The amount

of extracellular water was calculated in each case by dividing the amount of NaCNS injected by the concentration of thiocyanate in the serum water, making allowance for any thiocyanate excreted in the urine during the one hour interval between the times of administration of NaCNS and the collection of the blood sample. In addition to the 3 estimations obtained during the control periods in dogs 5, 6 and 7, three measurements of extracellular water were made in normal dogs 9, 10 and 11 and these results are included in table 3. The average of 6 estimations of the extracellular water in normal dogs was 30.7 ± 1.4 per cent of the body weight.

During ketosis in the depancreatized dogs 5 and 6 the percentage of extracellular water was increased by 12.5 and 8.4 per cent respectively. It would seem noteworthy that in both of these animals the actual quantity of extracellular water present during this period was practically the same as during the control period. In dog 7 the percentage of extracellular water was increased 3.7 per cent during starvation.

From the data in table 3 it will also be observed that the percentages of solids contained in rectus muscle during ketosis in dogs 5 and 6 were only slightly less than those obtained during the control period,—the percentages of muscle water thus being only slightly increased during ketosis.

DISCUSSION. It would seem noteworthy that our findings during ketosis in depancreatized dogs of increased serum water and extracellular water per kilo of body weight are the opposite to similar measurements obtained in adrenal cortical insufficiency. In the latter condition the serum volume (12) and extracellular fluids are diminished (13) and the studies of Harrop and others suggest that the percentage of intracellular water is increased. Swingle and his associates (14) concluded that the intracellular fluids are increased in experimental adrenal insufficiency and that following the administration of cortin to adrenalectomized dogs the accumulation of intracellular fluid and electrolytes shifts from the intracellular to the extracellular and vascular compartments.

The question may be raised as to whether our observations during ketosis in depancreatized dogs are similar to those obtained during severe ketosis in diabetes mellitus. Certainly the changes in concentrations of the serum components are analogous to those obtained in humans. It is difficult to evaluate degrees of dehydration in dogs; however, these animals presented dry mucous membranes and desired to ingest large amounts of water. It should be noted that they had free access to water at all times.

Our data do not permit calculation of the water in the intracellular fluid compartment. However, since the animals at the time of our measurements during ketosis appeared dehydrated an inference might be drawn that, if the body water per kilogram of body weight were diminished, water must have been lost from the intracellular fluid compartment.

Chang, Harrop and Schaub's observations of decreased circulating

blood and plasma volumes during ketosis in diabetes mellitus may not necessarily be inconsistent with our observations. These workers reported diminution in the total blood and plasma volumes in 5 individuals with severe acidosis when compared to the values obtained during recovery. It would seem possible that the values for blood volume obtained during recovery in their patients might not necessarily represent the values that might have been obtained after they were controlled for a longer period or at a time when they had not been suffering from diabetes mellitus.

SUMMARY

Measurements of the serum volume, extracellular fluid and various serum components were made in totally depancreatized dogs that had been permitted to go into ketosis following the withdrawal of insulin.

The serum volume per kilogram of body weight in our animals was found to be consistently increased during ketosis. The total volumes of serum in the circulation were, however, decreased when compared to the total volumes measured during the healthy state. Although the concentrations of electrolytes were consistently diminished during ketosis, nevertheless, owing to the increase in serum volume per kilo of body weight, the total amounts of the serum electrolytes (excepting $BHCO_3$) per kilogram of body weight were found to be actually increased. Our studies also indicated that during diabetic ketosis the extracellular water per unit of body weight was increased.

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THE EFFECT OF HYPOXEMIA ON VENTILATION AND CIRCULATION IN MAN

ERLING ASMUSSEN AND HUGO CHIODI[1]

From the Fatigue Laboratory, Harvard University, Boston, Mass.

The study of the factors controlling respiration and circulation has made much progress in the last two decades. Hering (1927), Koch (1933) and Heymans (1933) have opened new ways of investigation and made possible a more detailed study of the effect of carbon dioxide, oxygen and hydrogen-ion on both ventilation and circulation. Grollman's introduction of acetylene in cardiac output determinations made the original Krogh-Lindhard method easier to use and Christensen (1937) has shown that it remains the most reliable and handy method for use on human subjects.

All the reviews of the control of *respiration* (Nielsen (1936), Heymans and Bouckaert (1939), Gesell (1939) and Schmidt and Comroe (1940)) emphasize the dominant rôle of the tensions of the CO_2, of the O_2 and the H-ion concentration. It has not yet been possible to isolate as satisfactorily factors which control the *circulation* but evidently the same three also play very important rôles (Grollman (1932), Christensen (1937), McDowall (1938), Heymans and Bouckaert (1939) and others). It is the purpose of this paper to describe the effects of oxygen deficiency on the respiration and the circulation of normal men in rest and during work.

Oxygen deficiency has been evoked in two radically different ways, one by replacing part of the HbO_2 with HbCO, the other by letting the subject breathe a gas mixture low in oxygen. The data will be interpreted and discussed in the light of present hypotheses regarding the regulation of ventilation and circulation. For the sake of clearness, respiration and circulation will be treated separately.

A. RESPIRATION. Oxygen-lack evokes hyperventilation as pointed out by Haldane and Priestley (1905). This has been confirmed many times since then, and the mechanism of its effect on the ventilation has been carefully studied by, for instance, Lindhard (1911), Barcroft (1925), Nielsen (1936), Gesell (1939), and many others. The introduction of Heymans' technique again stimulated research in this field: numerous reports have been published on the effect of O_2-lack on the carotid glomus (see Heymans and Bouckaert (1939) and Schmidt and Comroe (1940)).

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[1] Fellow of the Guggenheim Foundation.

Procedure and methods. For all experiments the subjects came fasting to the laboratory in the morning. In rest the subjects were lying on a bed, and no determinations were made during the first hour. We thus made sure that we got basal and easily reproducible conditions. In work (walking on a motor-driven treadmill) no determinations were made before a steady state was reached. The subject breathed through mouthpiece and valve, the expired air was collected and measured in a Tissot-gasometer and a sample of it analyzed on the Haldane apparatus. The total ventilation was reduced to 0°, 760 mm. Hg and dryness for calculating the metabolism, and to 37°, prevailing barometric pressure and complete saturation for estimating the ventilation per minute. Alveolar air samples were taken in rest by the Haldane-Priestley method, during work by means of the modified Lindhard procedure.

The CO was prepared from $HCOOH$ and H_2SO_4 and collected over water. For administration a measured amount (usually between 350 and 450 cc.) was introduced into a Roth-Benedict apparatus, which afterwards was filled with oxygen. The subject was connected with the apparatus in the ordinary way and rebreathed the mixture for 30 to 45 minutes. A refilling with oxygen was necessary after about 20 minutes. About 10 minutes after disconnecting the subject from the Roth-Benedict apparatus a sample of venous blood was drawn and the experiment on the CO-poisoned subject began. Immediately after the end of the experiment a second sample of blood was taken. The CO contents were determined on the Van Slyke apparatus after Van Slyke and Neill (see Peters and Van Slyke, p. 328, 1932). The CO-content of the blood during an experiment is given as the average of the two determinations made just before and just after the experiment. The oxygen capacity of the blood was estimated from the cell volume by means of the formula of Dill, Edwards and Consolazio (1937). Blood samples (venous or capillary) were drawn for blood-lactate determinations after Edwards (1938). In some cases direct pH measurements on arterialized capillary blood were made by means of the glass electrode by Dr. W. H. Forbes.

Most of the experiments were carried out on three subjects, E. A., 32 years, 171 cm., 69 kgm.; H. C., 32 years, 170 cm., 75 kgm.; and B. C., colored, 20 years, 170 cm., 64 kgm. Normal oxygen dissociation curves had previously been determined for each subject.

Results. The results of our experiments on the three subjects, E. A., H. C. and B. C., are averaged in table 1. Figure 1 gives a graphic representation of the average ventilations plotted against the oxygen intake. The individual determinations were so close to each other that a separate presentation of them in a figure was impossible.

In table 1 the ventilations are given at 37°, observed barometric pressure and saturation. It is evident that the ventilations in rest and during

TABLE 1

SUBJECT	STATE	REST Ventilation l./min.	l./O₂	REST Alveolar Air pCO₂ mm.Hg	pO₂ mm.Hg	REST Arterial O₂ vol. per cent	per cent sat.	REST Blood lactate mgm. per cent	LIGHT WORK Ventilation l./min.	l./O₂	LIGHT WORK Alveolar Air pCO₂ mm.Hg	pO₂ mm.Hg	LIGHT WORK Arterial O₂ vol. per cent	per cent sat.	LIGHT WORK Blood lactate mgm. per cent	HEAVY WORK Ventilation l./min.	l./O₂	HEAVY WORK Alveolar air pCO₂ mm.Hg	pO₂ mm.Hg	HEAVY WORK Arterial O₂ vol. per cent	per cent sat.	HEAVY WORK Blood lactate mgm. per cent
E. A.	Normal	4.7	19.7	42	96	17.9	96	12	21.6	20.8	41	100	17.9	96	10	38.6	21.3	45	99	17.9	96	13
	CO-poisoned	4.8	19.1	41	99	12.1	65	13	21.6	19.8	41	99	12.7	68	13	39.4	22.4			13.0	70	24
	Breathing low O₂ mixture	8.0	35.6	34	36	12.6	68		33.3	32.3	29	40	13.8	74	14	61.0	38.1	30	45	14.9	80	53
H. C.	Normal	6.0	27.1	43	101	18.2	96	12	26.9	22.8	37	106	18.2	96	10	41.9	22.7	44	98	18.2	96	21
	CO-poisoned	6.5	29.4	43	97	12.6	66	9	25.0	22.1	41	103	13.0	68	16	62.0	31.0	32	117	13.3	70	81
	Breathing low O₂ mixture	8.8	37.6	33	38	13.3	70		35.8	34.8	28	46	15.4	81	28	84.4	52.8	25	52	16.5	87	107
B. C.	Normal	6.4	27.2	40	102	19.2	96	12	26.3	26.8	34	107	19.2	96	12	35.6	23.7	34		19.2	96	15
	CO-poisoned	6.0	24.8	41	106	12.7	63	12	25.0	25.8	35	105	14.6	73	12	35.7	25.3	35		14.0	70	36
	Breathing low O₂ mixture	9.8	43.5	30	38	13.0	65															

TABLE 2

SUBJECT	STATE	REST O₂/min. l.	A-v. O₂-diff. cc./l.	Cardiac output l./min.	Pulse rate	Stroke vol. cc.	LIGHT WORK O₂/min. l.	A-v. O₂-diff. cc./l.	Cardiac output l./min.	Pulse rate	Stroke vol. cc.	HEAVY WORK O₂/min. l.	A-v. O₂-diff. cc./l.	Cardiac output l./min.	Pulse rate	Stroke vol. cc.
E. A.	Normal	0.239	47	5.1	59	86	1.04	92	11.3	99	114	1.81	109	16.6	120	138
	CO-poisoned	0.247	44	5.6	64	87	1.09	81	13.5	128	105	1.76	102	17.3	159	109
	Breathing low O₂ mixture	0.225	24	9.4	73	129	1.03	47	21.9	132	166	1.60	54	29.6	159	186
H. C.	Normal	0.221	52	4.3	58	74	1.18	103	11.5	105	110	1.85	128	14.5	150	97
	CO-poisoned	0.221	48	4.6	71	65	1.13	94	12.0	146	82	2.00	129	15.5	186	83
	Breathing low O₂ mixture	0.234	29	8.1	76	107	1.03	67	15.4	149	103	1.60	86*	18.6	184	101
B. C.	Normal	0.235	58	4.1	57	72	0.98	101	9.7	91	107	1.50	121	12.4	134	93
	CO-poisoned	0.242	52	4.7	68	69	0.97	102	9.5	120	79	1.41	119	11.9	174	68
	Breathing low O₂ mixture	0.225	23	9.8	71	138										

* Only two determinations, 85 and 87, both too high; cardiac output and stroke volume accordingly too low.

light work in all three subjects are virtually unaffected by CO hypoxemia. In the heavy work the same is the case in E. A. and B. C., whereas H. C. shows a rather large increase. The last column shows that this subject alone had a significant increase in blood lactate during this type of work (to 81 mgm. per cent). In low oxygen without CO-poisoning all three subjects showed marked increases in ventilation in rest. Both E. A. and H. C. could perform the work long enough to reach a steady state, also in low oxygen, and both show important increases in ventilation and—in the heavy work—in blood lactate. The third subject, B. C., could not continue work in low oxygen long enough to get any reliable determinations of his ventilation.

The alveolar CO_2 and O_2 pressures are normal in the CO experiments, except in the case of H. C. during heavy work, where the blood lactate was increased. In low oxygen (about 10 per cent O_2 in the inspired air) the pCO_2 dropped from around 40 mm. Hg to around 30 mm. Hg.

The arterial oxygen content in the control experiments is calculated on the assumption that the arterial blood is 96 per cent saturated with oxygen. In the CO experiments the "available Hb" is found by subtracting the amount bound to CO, as found by the Van Slyke analyses, from the total Hb. Of the "available Hb" 96 per cent is assumed to be in the oxygenated form. For the low O_2 experiments the oxygen saturation is derived from the individual oxygen dissociation curves of the subjects, using the alveolar pO_2 as the O_2 tension of the arterial blood. It will be seen that the oxygen content of the arterial blood was decreased to approximately the same degree in the resting experiments under CO and with low O_2; in the work experiments the oxygen content usually was less decreased in low O_2 than in the CO experiments.

Figure 1 shows that the increase in ventilation with increasing oxygen consumption takes place in practically the same way in normal subjects and in subjects where 20 to 30 per cent of the Hb is saturated with CO, whereas the ventilation in low O_2 starts at a higher level and increases faster than in the two other series.

Discussion. Our results show that there is a markedly different respiratory response to the two forms of hypoxemia investigated, despite the fact that in each case 20 to 30 per cent of the hemoglobin is unsaturated with O_2. It is reasonable to assume that in all tissues where oxygen is used—e.g., in the muscles, the heart, and in the brain—about the same initial degree of oxygen deficiency must have occurred. A hypoxemia can, of course, be counteracted by an increase of the blood flow, but—as will be shown later—the blood flow is not accelerated in the CO experiments, whereas in low O_2 it is considerably increased. The oxygen deficiency of the tissues—including the brain—consequently should be more severe in the anemic hypoxemia (CO-experiments) than in the hypoxic

hypoxemia (low O_2-experiments), and nevertheless it is in the last case we find an increase in the ventilation. General oxygen-lack in the tissues—muscles, brain, etc.—therefore cannot be assumed to cause the hyperventilation.

This result is in good agreement with the experiments upon animals, where it often has been demonstrated that perfusion of the brain with hypoxic blood does not produce any hyperventilation, but, on the contrary, frequently a hypoventilation (Heymans and Bouckaert, Schmidt and Comroe). Quite different is the result when the *carotid body* is perfused with hypoxic blood: in that case a marked hyperventilation is always found, and the importance of the carotid body as an accessory to the respiratory center is now a well-established fact. It is therefore quite
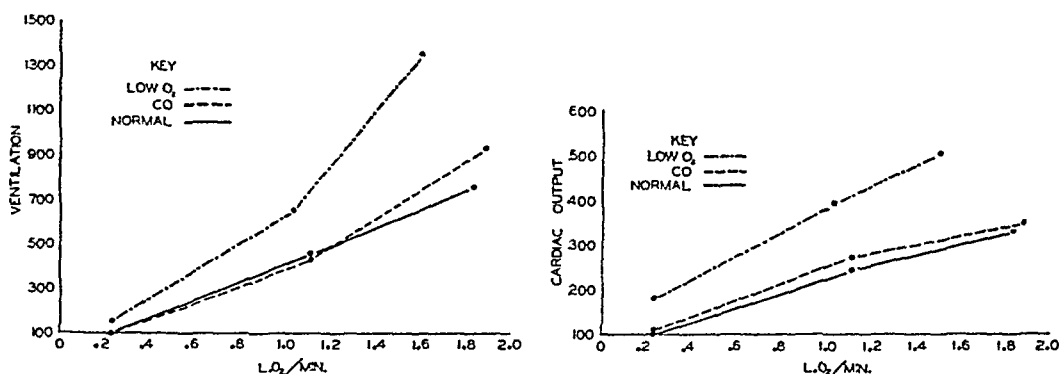
Fig. 1. Left. The ventilations of E. A. and H. C. averaged and plotted against the O_2-consumption. The ventilations are shown as percentages of the normal, resting value.

Fig. 2. Right. The cardiac outputs of E. A. and H. C. averaged and plotted against the O_2-consumption. The cardiac outputs are shown as percentages of the normal, resting value.

natural to assume that the effect of the low oxygen also in man is evoked through the chemoreceptors of the carotid body.

The most common explanation of the way in which hypoxemia affects the chemoreceptors is to assume that it produces a local oxygen-lack in the cells of the glomus. This explanation can be maintained if we assume that the oxygen consumption of the glomus is low in relation to the arterial supply, which we know is abundant, so that the reductions in combined O_2 and hence in pO_2 are small. This means that in CO-poisoning the pO_2 within the glomus is not much below its normal values. When the pO_2 of arterial blood supplied the glomus is low—as when a low O_2 mixture is breathed—the pO_2 within the glomus will be even lower, and there is a strong stimulus transmitted to the respiratory center.

Table 1 shows that in work experiments where the blood lactate was increased beyond about 50 mgm. per cent the ventilation was increased

out of proportion to the increase in metabolic rate. However, it is not possible to draw any conclusions from our data as to the effect of lactate accumulation on the ventilation except this simple statement. It might, though, be of interest to note that in the very few cases in which direct pH determinations on arterialized capillary blood were made (during work in low O_2) the pH was found to be normal or increased, even when the blood lactate was as high as 53 mgm. per cent. The acid effect of lactate accumulation must have been balanced or out-weighed by the buffer action of proteins, and the elimination of extra carbonic acid by hyperventilation.

B. CIRCULATION. The effect of hypoxemia on the circulation has been widely studied. Most observations, however, have been made on the pulse rate and on blood pressure, rather few on the circulation rate. Hasselbalch and Lindhard (1915) made some determinations of the cardiac output in a low pressure chamber by means of the nitrous-oxide method of Krogh and Lindhard but found no increase in the circulation rate, presumably because the O_2 tension was not low enough. Douglas, Haldane, Henderson and Schneider (1913) and Barcroft and co-workers (1923) found no effect of high altitude on the cardiac output, but their methods were inferior to those used later. Ewig and Hinsberg (1930) and Grollman (1930) found an increase of the cardiac output in the first days of a sojourn in high altitude, as did Christensen and Nielsen (1936) and Christensen and Forbes (1937) both in a low pressure chamber and in high altitude. The general result of the present observations in low O_2 seems to be the one set forth by Christensen and Forbes: There is an initial increase of the cardiac output when the O_2 tension is low enough, but prolonged (i.e., lasting several days) exposure is followed by a secondary return of the cardiac output to normal sea level values.

A few observations on anemic hypoxemia (e.g., H. E. Nielsen (1934) on man) indicate that prolonged anemia at least is accompanied by an increased cardiac output.

As there seems to be a difference between the effects of acute and more prolonged hypoxemias on the cardiac output, it might be worth mentioning that the present paper deals only with the acute forms of hypoxemia.

Procedure and methods. The subjects were the same as in the previously described experiments, and so were the precautions taken to secure reproducible conditions. Pulse rates were obtained in rest by counting the pulse at the wrist for half a minute, in work by counting the clicks of a cardiotachometer, which picked up and amplified the action potential of the heart. Blood pressures were measured in rest at the left upper arm by the ordinary auscultatory method. The cardiac output was estimated from the rate of the O_2 consumption and the arteriovenous O_2-difference, which was obtained by means of Christensen's modification of

Grollman's acetylene method. When used with the prescribed precautions (see Christensen (1937) for details) this method gives reliable results in rest and in work, when the alveolar O_2 tension is normal or above normal. When the alveolar O_2 tension is lower than normal, special precautions must be taken to make sure that the average O_2 tension in the gas samples taken during the determination is about the same as the alveolar O_2 tension observed beforehand (Lindhard, 1915; Grollman, 1930; Christensen and Nielsen, 1936). Grollman (1930) suggests a correction for deviations from this rule by means of the oxygen dissociation curve or by interpolation, and Christensen and Nielsen (1936) think that a determination of the oxygen intake during the rebreathing period will give a control of the reliability of the determination. In our opinion, neither of these methods of correction is reliable. We therefore prefer, in the experiments with low O_2, to use only those values of the a-v O_2 difference during the determination of which the average O_2 tension was equal to, or very close to, the alveolar O_2 tension. The procedure in the low O_2 experiments, therefore, was first to secure a sample of alveolar air, as described before; then, after some minutes, to let the subject exhale deeply, have him quickly change over to the mouthpiece of the Grollman bag, and then to perform the experiment in the usual way. In the experiments with partial saturation of the Hb with CO, no such precautions are necessary, as the available Hb in this case will be completely saturated with O_2 in passing the lungs.

Results. Table 2 and figure 2 show the average data of our experiments. It will be seen that the a-v O_2 differences in normal conditions, and with about 30 per cent HbCO in the blood, both in rest and during work are very similar, whereas the a-v O_2 difference in low O_2 is greatly diminished. Accordingly, the cardiac output with CO is only slightly increased, whereas in low O_2 it is significantly increased. The pulse rates do not correspond to this difference: high pulse rates are found both with CO and in low O_2, which demonstrates the well-known fact that the pulse rate cannot always be taken as an indicator of the rate of the blood flow. The stroke volume shows a very interesting fact: it is lower than normal with CO, equal to or higher than normal in low O_2. The blood pressure, measured only in rest, was approximately normal in low O_2 and showed a slight tendency to an increase with CO.

It is necessary, during the discussion, to mention the average capillary O_2 tension. A direct measurement of this has not been made, but it can be assumed to have been close to the average venous O_2 tension. An estimation of this is shown for one of the subjects in table 3. In table 3 the arterial O_2 content in volumes per cent is taken from table 1 and the arterial O_2 tension is assumed to equal the alveolar O_2 tension in the same table. The venous O_2 content is determined by subtracting the a-v

O_2 difference of table 2, expressed in volumes per cent, from the arterial O_2 content, and the venous O_2 tension is estimated from O_2 dissociation curves of the arterial blood, normal and with 30 per cent of CO saturation (formula in Peters and Van Slyke, p. 618). This introduces an error, as dissociation curves of *venous* blood ought to be used. The difference, however, would even in work be less than 5 mm. Hg, so that for our purpose this approximation will serve. Table 3 shows that whereas the venous O_2 tension in low O_2 is approximately normal, it is very low in the CO experiments.

Discussion. The experiments, the results of which are presented in table 2 and figure 2, show that the two forms of hypoxemia—the anemic in CO-poisoning and the hypoxic in low O_2—interfere with the circulation in different ways.

The only effects of the anemic hypoxemia on the circulation are apparently a high pulse rate and a correspondingly low stroke volume (see

TABLE 3

SUBJECT	STATE	REST				LIGHT WORK				HEAVY WORK			
		Arterial O_2		Venous O_2		Arterial O_2		Venous O_2		Arterial O_2		Venous O_2	
		Vol.	Tension	Vol.	Tension	Vol.	Tension	Vol.	Tension	Vol.	Tension	Vol.	Tension
		per cent	mm. Hg	per cent	mm. Hg	per cent	mm. Hg	per cent	mm. Hg	per cent	mm. Hg	per cent	mm. Hg
E. A.	Normal	17.9	96	13.2	37	17.9	100	8.7	28	17.9	99	7.0	25
	CO-poisoned	12.1	99	7.3	16	12.7	99	4.6	11	13.0	100	2.8	7
	Breathing low O_2 mixture	12.6	36	10.2	30	13.8	40	9.1	29	14.9	45	9.5	29

table 2). The cardiac output is practically normal. The increase in pulse rate can be looked upon in at least two different ways: First, it could be assumed to be an unsuccessful attempt by the regulatory mechanisms of the body to increase the circulation. Second, it could be assumed to be part of a regulatory mechanism which keeps the cardiac output from falling below the normal. Against the first assumption speaks the fact that the cardiac outputs in the CO experiments with all three subjects, in rest and during work, are so near normal. This would be a very extraordinary coincidence if the aim of the pulse acceleration were to increase it beyond normal. The second assumption, that the pulse rate is high in order to keep the cardiac output from falling below normal, makes necessary the assertion that the cardiac output would have been subnormal if the pulse acceleration had not taken place. As the cardiac output is the product of pulse rate and stroke volume, this means that the stroke volume of the heart, for one reason or another, is abnormally low in the CO ex-

periments. A subnormal stroke volume is ordinarily the effect of a subnormal venous pressure, which again can be caused by an abnormal distribution of the blood in the vessels. In parallelism to this, it is quite natural to assume that the low stroke volume in the CO hypoxemia is caused by a decreased filling of the central veins, called forth by a pooling of blood in the capillaries, which no doubt at the prevailing low capillary pO_2 (comp. table 3) will be widely dilatated (Krogh, 1929). A low venous pressure would result in lowering the arterial blood pressure, were it not for a pressosensible reflex from the aortic arch and the carotid sinus that compensates for the falling blood pressure by an increased pulse rate. (For a more detailed discussion, see Asmussen, Christensen and Nielsen, 1939, 1940.)

The increased pulse rate in the anemic hypoxemia of CO-poisoning thus can be explained as a pressoregulatory compensation for the vasodilatation. A stimulation of the circulatory center, in an attempt to compensate for the low tissue O_2 tension which caused the vasodilatation, has apparently not taken place. It is therefore reasonable to conclude that acute O_2-lack in the tissues is, neither directly nor indirectly, i.e., via the pressoregulation, a stimulus for the circulatory center.

Quite different is the picture when, as in the hypoxic hypoxemia, the O_2 tension of the arterial blood is low. In this case we find a greatly increased cardiac output, effected by both a pulse acceleration and an increased stroke volume (table 2). The low O_2 content of the arterial blood is compensated for by the increased blood flow, so that the capillary O_2 tension, as indicated by the venous O_2 tension in table 3, remains normal. It is obvious that the low O_2 tension of the arterial blood must have acted as a stimulus on the mechanisms which regulate the circulation.

From experiments on animals it is now well known that low arterial O_2 tension produces pulse acceleration and hypertension if the chemoreceptors of the carotid body are intact, whereas no effect is obtained if the chemoreceptors are denervated (Schmidt and Comroe, 1940). In man presumably the same mechanisms are at hand, so that it is reasonable to assert that the effect of the low arterial O_2 tension on the cardiac output in man is produced via the chemoreceptors of the carotid (and aortic) body.[2]

It is emphasized that this paper has dealt only with *acute* hypoxemias, i.e., hypoxemias lasting only up to a few hours. Prolonged exposures will have quite different effects, as the results from prolonged exposures to low O_2 indicate (Grollman, 1930; Christensen and Forbes, 1937), and as the investigations on patients with pernicious anemia have shown (Nielsen,

[2] The ways in which an increase in cardiac output actually is produced—by emptying the blood depots, pulse acceleration and compensatory vasoconstrictions in idle organs—have been discussed by Krogh (1912) and will not be considered here.

1934).   The ways in which this adaptation is effected need further investigation.

SUMMARY

A. Respiration.   Our experiments have shown that hypoxemia caused by partial CO-poisoning (20-30 per cent) (anemic hypoxemia) elicits little or no respiratory response whereas hypoxemia caused by an alveolar pO_2 of about 40 mm. Hg (hypoxic hypoxemia) causes a very pronounced hyperventilation both in rest and in work.

It is concluded: 1. General O_2-lack in the tissues does not evoke a hyperventilation.   2. The O_2 *tension* of the arterial blood, and not its O_2 *content*, is the active factor in causing hyperventilation.   3. The effect is produced through the pO_2 of the carotid body (glomus).

B. Circulation.   Our experiments have shown that both in rest and during work a state of *hypoxic hypoxemia*, with the O_2 in the inspired air low enough to reduce the arterial HbO_2 to 70 or 80 per cent, produces an increase in cardiac output above the normal.   Furthermore, both in rest and during work a state of *anemic hypoxemia* (20-30 per cent Hb saturated with CO) has little or no effect on the cardiac output, but increases the pulse rate considerably.

It is concluded: 1. Acute O_2-lack in the tissues is not a stimulus for the circulation.   2. A lowered O_2 *tension* of the arterial blood, not a lowered O_2 *content*, is a stimulus for the circulation.   3. The effect probably is produced through the chemoreceptors of the carotid glomus.

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PARASYMPATHETIC SENSITIZATION IN THE CAT'S EYE

FRANCIS C. KEIL, Jr.[1] AND WALTER S. ROOT

From the Department of Physiology, College of Physicians and Surgeons, Columbia University

In 1921 Loewi (1) discovered that vagal stimulation of a frog's heart liberated a substance which when perfused through another frog's heart induced typical vagal inhibitory effects. This finding was of considerable interest, for it had been noted that acetylcholine mimics the action of the parasympathetic nerve impulses (2). Much experimental evidence suggesting the identity of Loewi's "vagusstoff" and acetylcholine has accumulated (3). The action of this drug can be intensified either by previous treatment of the tissue with physostigmine or by parasympathetic denervation. Under these conditions the cat's pupil constricts strongly when small amounts of acetylcholine are instilled into the conjunctival sac (4), or injected into the anterior chamber of the eye (5).

The following study was designed to investigate the responses of the pupil to acetylcholine after interruption of the pre- and post-ganglionic parasympathetic nerve fibers.

METHODS. All operations were carried out with strict aseptic precautions on cats anesthetized with nembutal (36 mgm. per kgm. body weight, intraperitoneally). The parasympathetically decentralized pupil was obtained by intracranial section of the oculomotor nerve. The nerve was approached by trephining the parietal bone and raising the temporal lobe of the brain from the middle fossa. Denervation of the sphincter was accomplished by excision of the ciliary ganglion (4). No ocular muscle was cut, nor were any of the intraorbital vessels injured. Anderson's observation (6) that certain animals develop a slight opacity of the cornea which gradually disappears was confirmed. The region involved coincided with that part of the cornea which was uncovered when the lids were partly closed. In the later operations corneal injury was avoided by closing the eyes with adhesive tape during the period of anesthesia.

The responses of the circular muscle of the iris to acetylcholine were determined at various intervals after operation. Since the instillation of acetylcholine (1 to 5 per cent) produces an inflammation of the con-

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[1] Resident at the New York Eye and Ear Infirmary.

junctiva, the drug was injected intravenously.   The injection of less than
2 mgm. per kgm. of body weight produced slight effects on the sensitized
sphincter of the pupil.   To obtain constriction consistently, doses of 5
mgm. per kgm. were required.   Unless specifically mentioned, no physo-
stigmine was instilled during the sensitization tests.   During the acetyl-
choline tests the cats were not anesthetized.

Changes in pupillary size were usually determined by measuring the
horizontal diameter with a millimeter scale.   In certain instances the eye
was photographed with a synchronized flash light attached to the camera.
Lasting but $\frac{1}{75}$ of a second, the flash was well within the latent period of the
light reflex (7).

RESULTS.   Immediately following intracranial section of the oculomotor
nerve (5 cats) or excision of the ciliary ganglion (8 cats), the ipsilateral
pupil was markedly dilated.   Twenty-four to thirty-six hours after either
operation examination of the animals revealed a prominent bulbus despite
the presence of a slight ptosis.   The direct light reflex was absent but light
directed into the dilated pupil produced a consensual pupillary constriction.
Inconstant muscle palsies were produced by section of the oculomotor
nerve.   In two cats which had suffered section of the third cranial nerve,
the size of the pupil varied slightly during the succeeding two or three
days (Anderson, 6).

Five cats in which the oculomotor nerve was sectioned showed a tem-
porary loss of the contralateral contact placing reactions.   One animal
which lived for five days was never able to walk.   Since in all cases a beam
of light directed into the dilated pupil produced constriction of the opposite
sound pupil, functional integrity of the ocular reflex centers may be
assumed.

The normally innervated cat eye was but little affected by the intra-
venous injection of 5 mgm. of acetylcholine per kilogram of body weight.
Under the special condition of exposure to darkness, however, the pupil
showed a slight dilatation in response to the drug (fig. 1, photographs).
In general these results agree with the work of other investigators which
indicates that the intravenous injection of small doses of acetylcholine is
without effect (8), whereas the injection of large doses produces variable
responses, none of which appear to be very great (9).   Likewise, the
instillation of two drops of 5 per cent acetylcholine into the conjunctival
sac of the normal cat eye fails to induce pupillary constriction (4).   In the
animals in which a single operation was performed the pupil on the un-
operated side served as the control.

Within 24 hours after section of the third cranial nerve or the removal
of the ciliary ganglion, the intravenous injection of acetylcholine produced
a constriction of the operated pupil.   Indeed, the injection induced so
great a constriction of the extremely dilated pupil that it became smaller

than the contralateral control pupil (fig. 1, photographs). This is an example of the "paradoxical pupillary constriction" of Anderson (6). The magnitude of the response to acetylcholine was much greater by the fifth day and was maintained at this level until about the eighteenth day, when it began to diminish. It reached a steady minimum in approximately thirty-five days. Figure 1 illustrates this phenomenon in a cat which had suffered section of the right oculomotor nerve. Similar pupillary
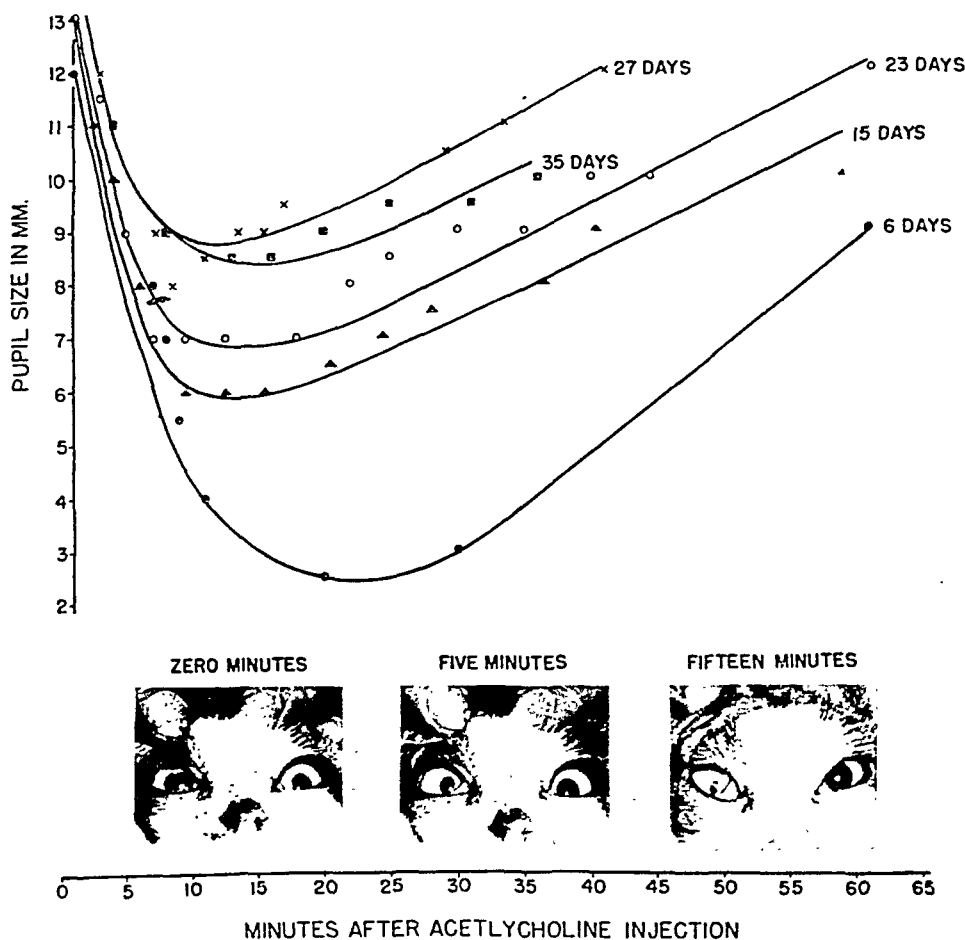
Fig. 1. The effect of intravenously injected acetylcholine (5 mgm. per kgm.) upon the size of the right pupil 6, 15, 23, 27 and 35 days after intracranial section of the right oculomotor nerve. Cat 1. The photographs refer to the six-day curve.

responses to acetylcholine after ciliary ganglionectomy are shown in figure 2. Constriction of the pupil on the side of the lesion was observed two to three minutes after injection. The constriction became maximal within seven to thirty minutes and gradually decreased during the succeeding half-hour. Pupillary constriction produced by acetylcholine injection, persisting long after the drug must have disappeared from the circulation, has been reported by Bender and Weinstein (8). It is important to note

that in our experiments the injection of acetylcholine produced an identical miosis whether the lesion interrupted pre- or post-ganglionic parasympathetic fibers.

To demonstrate that the diminished response to injected acetylcholine was related to the time which had elapsed since operation, bilateral ciliary ganglionectomy (2 cats) was carried out in two stages. Figure 3 shows that six days after the excision of the left ciliary ganglion and thirty-two days after the removal of the right ciliary ganglion the intravenous injection of 5 mgm. of acetylcholine per kilogram of body weight produced a
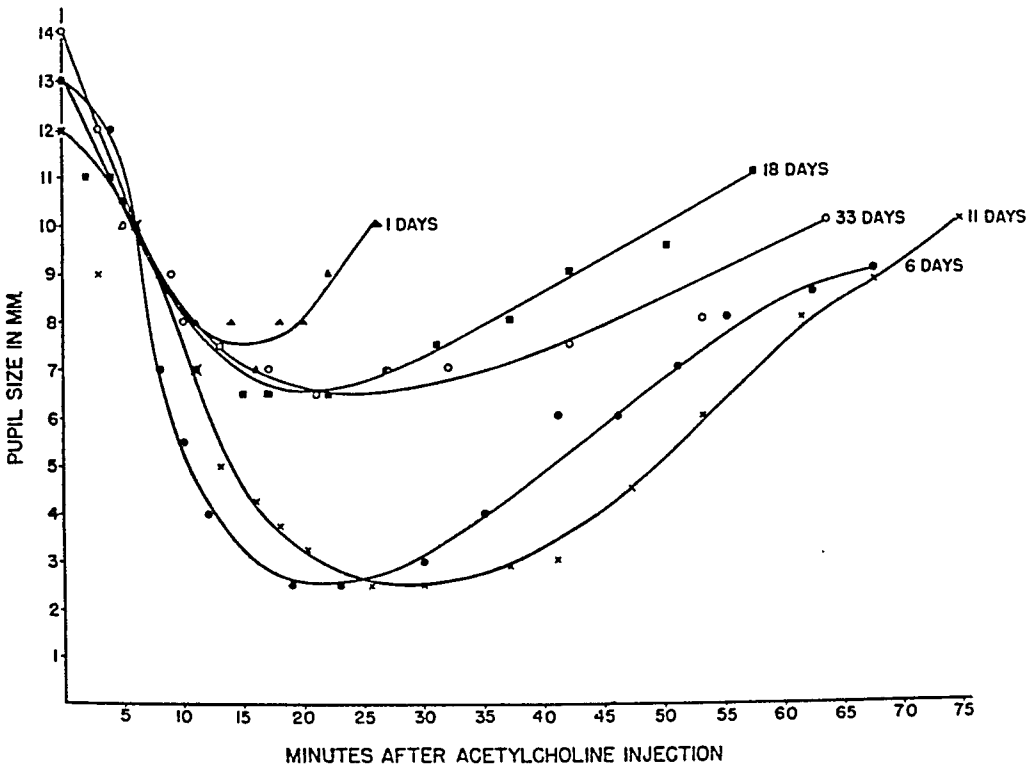
Fig. 2. The effect of intravenously injected acetylcholine (5 mgm. per kgm.) upon the size of the right pupil 1, 6, 11, 18 and 33 days after excision of the right ciliary ganglion. Cat 2.

constriction of both pupils. The more recently denervated sphincter showed a greater constriction than did that of the other side. The pupillary constriction curve produced by acetylcholine injection six days after excision of the left ciliary ganglion compares nicely with that obtained six days after removal of the right ciliary ganglion (compare figs. 2 and 3). These findings indicate that the time between the removal of the ciliary ganglion and the injection of acetylcholine constitutes an important factor in the magnitude of the pupillary response.

At various times after operation physostigmine was instilled into the

eyes of all of our animals.   Two drops of a 1 per cent solution of this drug
instilled into the conjunctival sac of the decentralized eyes produced the
expected constriction (10).   Several days after excision of the ciliary
ganglion physostigmine was without effect (5, 10, and others).   Instillation
of physostigmine followed twenty minutes later by acetylcholine injection,
in a cat sixty-two days after the removal of the left ciliary ganglion, induced
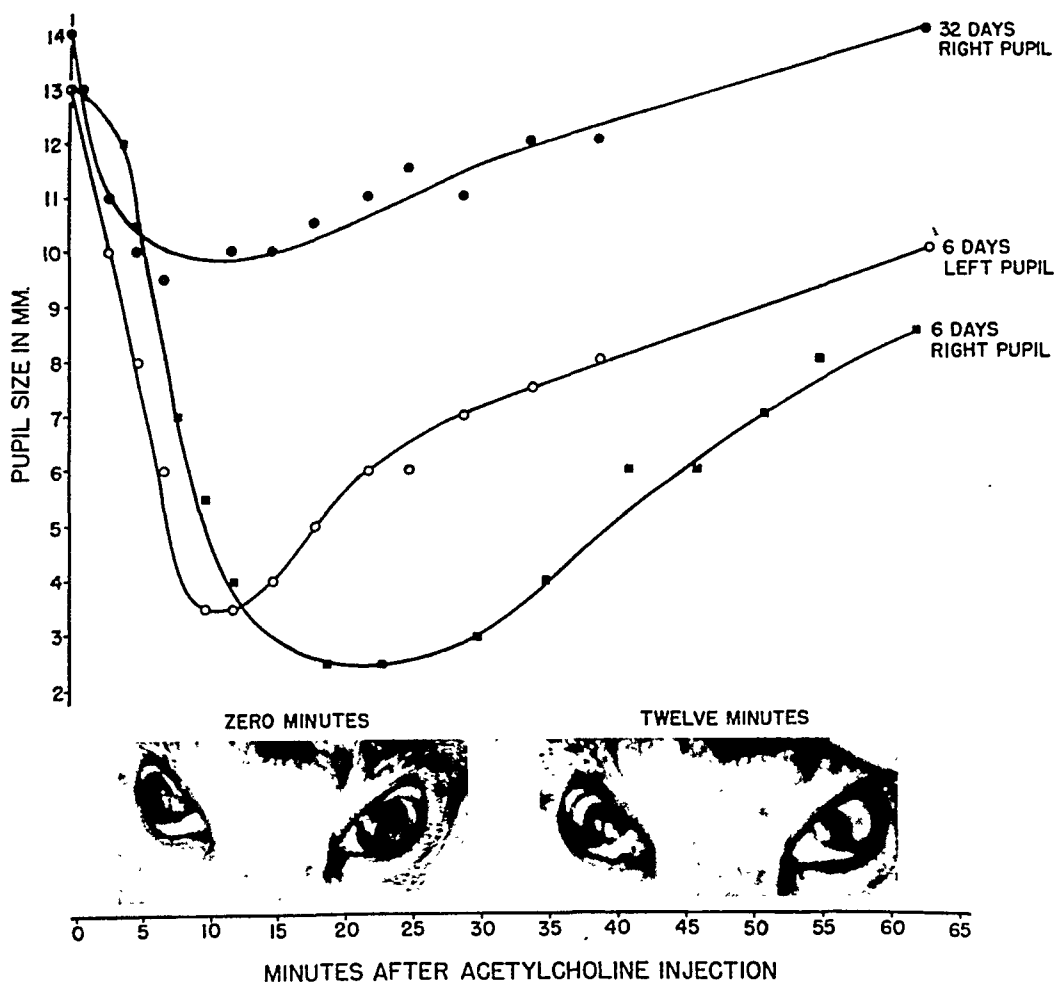
Fig. 3. The effect of intravenously injected acetylcholine (5 mgm. per kgm.)
upon the size of the pupils 32 days after excision of the right ciliary ganglion and 6
days after removal of the left ciliary ganglion.   The response of the right pupil
6 days after right ciliary ganglionectomy is included for comparison.   Cat 2.

a constriction of the left pupil comparable with that which had been present
eight days after operation (fig. 4).   In this cat the reaction to physostig-
mine alone was negative, and the acetylcholine tests twenty-two and
thirty-eight days after parasympathetic denervation showed that the
pupillary response had diminished.   The smaller pupillary response to
acetylcholine, shown by the right eye of cat 2 (fig. 3). was increased when

physostigmine instillation preceded acetylcholine injection. Under these conditions the magnitude of the constrictions of both pupils was equal to that shown by the right eye in figure 3. To prevent the rapid destruction of acetylcholine by choline esterase, Shen and Cannon (4) instilled eserine in most of their experiments. Had this procedure been used
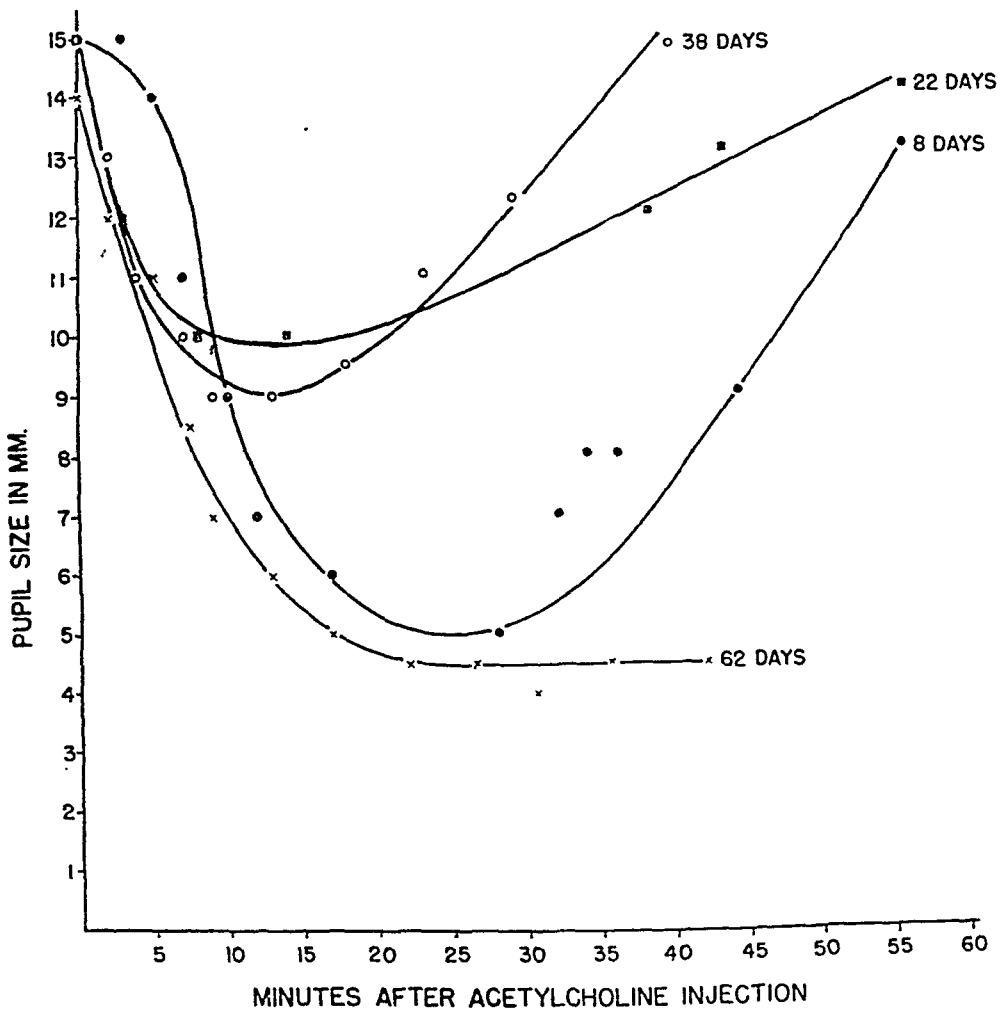
Fig. 4. The response of the left pupil to intravenously injected acetylcholine (5 mgm. per kgm.) 8, 22 and 38 days after left ciliary ganglionectomy. At 62 days 2 drops of 1 per cent physostigmine were instilled into the left eye followed 20 minutes later by the intravenous injection of acetylcholine (2.5 mgm. per kgm.). Physostigmine instillation alone was without effect. Cat 3.

routinely in our study the pupillary loss of sensitization to acetylcholine would have been missed.

Between two and three months after the excision of the ciliary ganglion the instillation of physostigmine alone into the conjunctival sac induced pupillary constriction. This observation confirms that of Anderson (10)

who noted a positive reaction in cats two months after ciliary ganglionec-
tomy. According to Anderson the regenerating fibers come not only
from the preganglionic stump, but also from motor branches of the ocu-
lomotor nerve to the extrinsic ocular muscles. This is understandable on
the basis of the general rule that cholinergic fibers are interchangeable
functionally with other cholinergic fibers (11). The accessory ciliary
ganglia (10, 12) may also serve as a source of regenerating fibers. In
agreement with Anderson, a positive reaction to physostigmine was ob-
served at a time when no return of the light reflexes had occurred. It must
be noted that the reaction to physostigmine was negative during the period
of diminishing sensitivity to acetylcholine.

The rate of regeneration following section of the oculomotor nerve was
highly variable. In one cat the pupil on the side of the lesion became less
dilated, and twenty-nine days after operation it responded to light. Simi-
lar phenomena were shown by a second animal on the forty-seventh day.
Two other cats which survived for long periods had shown no signs of
regeneration at the end of fifty-nine and sixty-five days, respectively.
These observations, confirming earlier studies of oculomotor nerve regen-
eration (6, 10), indicate that functional regrowth of the severed nerve
fibers had not taken place during the period of diminished sensitivity to
acetylcholine.

Marked systemic reactions were observed immediately after the intra-
venous injection of acetylcholine. The animal usually attempted to run,
then fell upon its side in clonic and tonic convulsions. In some cases a
few seconds of artificial respiration was applied; within five minutes the
cat had recovered sufficiently to sit up. Profuse salivation, lachrymation,
sweating of the foot pads, and dyspnea were present for about ten minutes.
Urination and defecation occurred in certain animals. The systemic
effects of acetylcholine injection were of greater intensity when instillation
of physostigmine preceded the injection. Indeed, the injection of 5 mgm.
of acetylcholine per kilogram of body weight killed one cat although this
dose had been tolerated several times in the absence of physostigmine.

DISCUSSION. Following the intravenous injection of adequate amounts
of acetylcholine, contraction of the decentralized and denervated sphincter
of the iris occurred. The secondary dilatation of the pupil induced by
acetylcholine injection which has been reported by Bender and Weinstein
(8) was not observed. Since these authors worked with cats having com-
pletely denervated pupils, it must be supposed that in the presence of
sympathetic innervation secondary dilatation does not occur.

No apparent difference in the responses of the pupil to acetylcholine
after pre- or post-ganglionic interruption of the parasympathetic fibers
was observed. This is not surprising, for the post-ganglionic as well as
the pre-ganglionic parasympathetic fibers are regarded as cholinergic (11).

The results of this study indicate that pupillary sensitization to the intravenous injection of acetylcholine decreases in about three weeks. That a systemic tolerance to the drug was not present is shown by the experiments in which the ciliary ganglia were removed at different times. Under these circumstances the more recently denervated sphincter showed a greater contraction which, however, followed the general contraction curve that the contralateral pupil had displayed previously. A second possible explanation is that excision of the ciliary ganglion might have injured the ocular sympathetic pathway in the orbit, and produced sensitization of the pupil to adrenin. Adrenin is known to be released by the direct action of intravenously injected acetylcholine upon the cells of the adrenal medulla (13, and others). This action, which might antagonize the pupillary response to acetylcholine, could account for the decrease in the observed parasympathetic sensitization. Such an explanation was excluded by demonstrating that the nictitating membrane of the eye from which the ciliary ganglion had been removed was not sensitized to injected adrenalin. This result agrees with the statement that the ocular sympathetic pathway does not pass through the ciliary ganglion in the cat (6, 12). That functional regeneration is not implicated was shown by the absence of the light reflexes, by the continued dilatation of the pupil, and, in the cats which had suffered ciliary ganglionectomy, by the negative responses to instilled physostigmine.

The acetylcholine sensitization described in this investigation could be explained by assuming that the interruption of parasympathetic fibers decreases the activity of the choline esterase system. Such an assumption is supported by the reports that section of the autonomic innervation of certain tissues decreases the choline esterase content (14, 15) and activity (15, 16). Moreover, ciliary ganglionectomy is said to result in the disappearance of acetylcholine from the iris and ciliary body (17). The experiments, in which physostigmine was first instilled into the eye, suggest that the diminished response to acetylcholine injection, occurring, as it did, three weeks or more after excision of the ciliary ganglion, is related to an increased activity of the esterase system. Under these circumstances pupils which had shown decreased responses to acetylcholine alone, now gave a maximal contraction to the injection of even small amounts of the drug.

In view of the above study, it is of interest to note that Pierce and Gregersen (18) injected 100 mgm. of acetylcholine into dogs two months after section of the chorda tympani and found no evidence of sensitization, as measured by the salivary flow. These authors suggested that sensitization may have been present at an earlier period.

On the basis of choline sensitization, Scheie (19) has placed the site of the disturbance of Adie's syndrome in the parasympathetic system at the

ciliary ganglion or peripheral to it. Our results, which show that acetylcholine sensitization follows section of the oculomotor nerve, do not support this conception.

SUMMARY

1. The normal pupil of the cat reacts only slightly to the intravenous injection of 5 mgm. of acetylcholine per kilogram of body weight.

2. Removal of the ciliary ganglion or intracranial section of the oculomotor nerve results in a marked acetylcholine sensitization of the iris sphincter. The magnitude of this sensitization is approximately the same whether the sphincter is denervated or decentralized.

3. Sensitization of the iris sphincter reached a maximum in about five days and similar curves could be obtained until about the eighteenth day. After this time the responses decreased until they reached a steady minimum within approximately thirty-five days.

4. The recovery of acetylcholine sensitization following the instillation of physostigmine suggests that the loss of sensitization is associated with an increased choline esterase activity.

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COMPARATIVE EFFECTS OF PROGESTERONE AND CORTICO-ADRENAL EXTRACTS ON NORMAL, ADRENALEC-TOMIZED AND OTHER ANIMALS[1]

E. L. COREY

With the technical assistance of R. KLINE

From the Physiological Laboratory of the University of Virginia Medical School

The work of Gaunt (1937) *et al.* (1938, 1939) has apparently established the ability of progesterone to maintain the life of the adrenalectomized ferret and has served to revive interest in the possibility of a direct inter-relationship between cortico-adrenal and ovarian secretion. Stewart (1913) first called attention to what he considered an unusual prolongation of the life-span of pregnant cats following adrenal removal, and later Stewart and Rogoff (1925), working with dogs, reported that pregnant animals survived adrenalectomy until the end of gestation. They suggested that the corpora lutea might be responsible for the unusually long survival periods observed. The writer (1928) was unable to substantiate these claims in experiments on an extended series of adrenalectomized pregnant and lactating cats.

Results on the rat. In the present study, comparisons were first made of the effects of cortico-adrenal extract and progesterone[2] on the carbohydrate metabolism of normal, fasted, adrenalectomized and hypophysectomized rats. Having long maintained that cortico-adrenal secretion is intimately concerned with carbohydrate metabolism (Corey, 1927, 1935; Corey and Britton, 1937, 1939), it was felt that such a comparison was essential to an evaluation of progesterone in the rôle of a substitute for cortico-adrenal secretion. The results are briefly presented in table 1. Figure 1 shows the results graphically.

Both table and figure indicate that progesterone, in the amount administered, did not influence carbohydrate metabolism in the rat. This was in distinct contrast to the marked rise in blood sugar and hepatic glycogenesis observed following cortico-adrenal extract administration.

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TABLE 1

Effects of cortico-adrenal extract and progesterone on carbohydrate metabolism in the rat

NUM-BER OF RATS	CONDITION OF ANIMALS	TREATMENT	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN
A. Animals in which all blood and tissue samples were taken 6 hrs. after the injections were made					
			mgm. per cent	*grams per cent*	*grams per cent*
3	Normal, fully fed	5 cc. cortico-adrenal extract	96	2.72	0.32
8	Normal, fully fed	5 mgm. progesterone	95	2.14	0.33
2	Normal, fully fed	1 cc. 0.9% NaCl	96	2.21	0.29
4	Normal, fasted for 24 hrs.	5 cc. cortico-adrenal extract	82	0.52	0.30
8	Normal, fasted for 24 hrs.	5 mgm. progesterone	66	0.09	0.24
2	Normal, fasted for 24 hrs.	1 cc. 0.9% NaCl	64	0.08	0.26
8	Adrenalectomized 4 days previous to experiment	5 cc. cortico-adrenal extract	90	0.71	0.30
7	Adrenalectomized 4 days previous to experiment	5 mgm. progesterone	64	0.24	0.31
2	Adrenalectomized 4 days previous to experiment	1 cc. 0.9% NaCl	66	0.21	0.33
B. Injections made every hour for 24 hrs., when blood and tissue samples were taken					
2	Hypophysectomized 7 days previous to experiment	1 cc. extract	98	0.72	0.30
4	Hypophysectomized 7 days previous to experiment	1 mgm. progesterone	76	0.15	0.16
2	Hypophysectomized 7 days previous to experiment	1 cc. 0.9% NaCl	75	0.13	0.15
C. Blood-sugar samples only					

NUM-BER OF RATS	CONDITION OF ANIMALS	TREATMENT	B.S. (INITIAL)	B.S. (2 HRS.)	B.S. (4 HRS.)	B.S. (6 HRS.)
			mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
3	Normal, fasted for 24 hrs.	5 cc. cortico-adrenal extract	74	99	92	73
3	Normal, fasted for 24 hrs.	5 mgm. progesterone	72	68	73	66

It was concluded that progesterone does not act similarly to cortico-adrenal extract in the rat since, unlike cortical preparations, it was wholly without carbohydrate influence in that animal.

Results on the cat. In a study of the action of progesterone in the cat, 21 animals were adrenalectomized and progesterone injection was begun either at once, or after symptoms of adrenal insufficiency had become plainly evident.
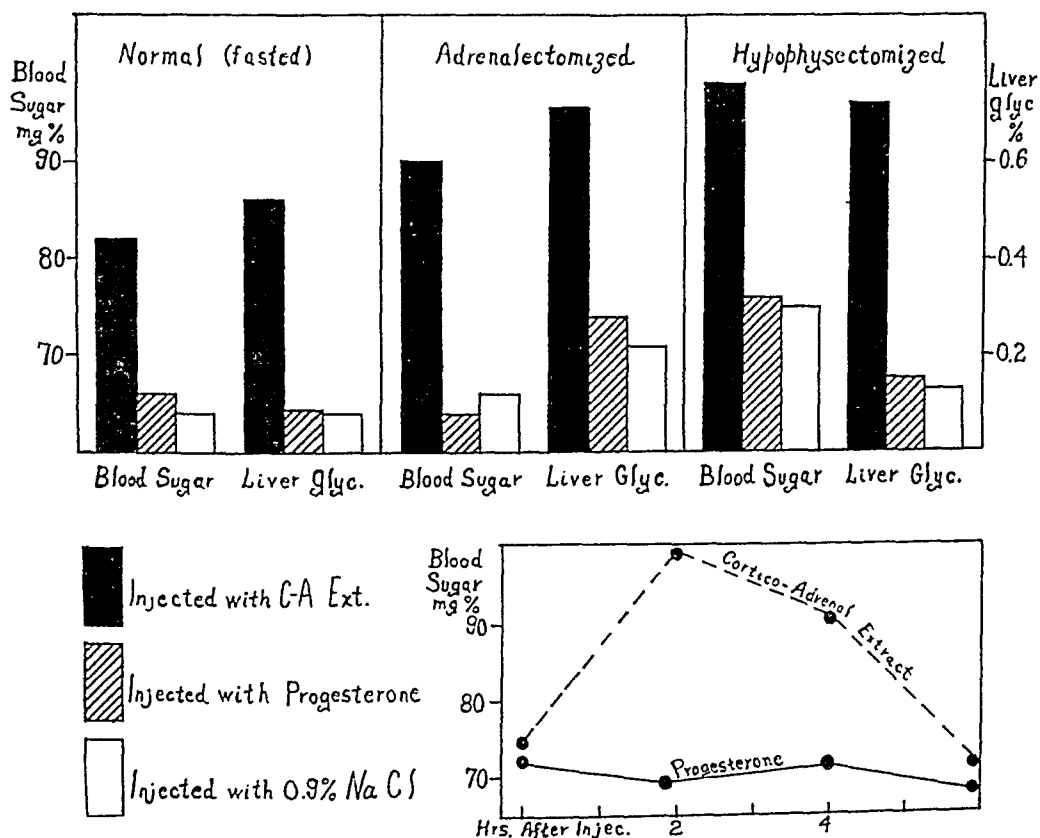
Fig. 1. Comparative effects of cortico-adrenal extract and progesterone on the blood sugar and liver glycogen levels of rats under various conditions.

Three male animals evinced symptoms of adrenal insufficiency (weakness, anorexia, ataxic gait) following bilateral adrenalectomy after an average of 4.5 days. Progesterone (5 mgm. twice daily) was then given until the animals were restored to an apparently normal condition, when the dosage was reduced to 5 or even 2 mgm. per day.

Two of the animals were maintained on progesterone for 21 days, this period being considered sufficiently long to indicate definite life-maintaining potency; also the large amounts of progesterone demanded by such experiments precluded more prolonged study. One animal was sacrificed while in apparent good health and the blood sugar, serum sodium, chloride,

potassium and urea levels were determined, as well as the liver, skeletal and cardiac muscle glycogen concentrations. All the analyses yielded results well within the normal range. The second animal was allowed to develop symptoms of adrenal insufficiency before progesterone administration was begun; it showed a definitely favorable reaction to the injections and was in good health after three weeks' treatment. Blood and tissue samples were taken 10 days after stopping injections, when the cat exhibited signs of profound adrenal insufficiency. Severe carbohydrate depletion, lowered sodium and chloride levels with uremia, and a high serum potassium concentration were present, i.e., the usual conditions found in cats suffering severe cortico-adrenal insufficiency. A protocol of observations on this animal, appended below, demonstrates the life-maintaining action of progesterone together with the glycemic response to injections obtained in this instance.

Protocol, cat 2. Male, weight 2.59 kilos. March 1, 1939, adrenalectomized (blood sugar 81 mgm. per cent).

March 6, 9:55 a.m. Weak, staggers. B.S. 66 mgm. 3:45 p.m. Much weaker. 10:00 p.m. Given 5 mgm. progesterone. Treatment begun (10 mgm. daily).

March 7 to 11, cat in excellent condition; appetite good; active. Average B. S. for this period 76 mgm. March 11, dosage reduced to 5 mgm. daily.

March 11 to 20, condition still good. Apparently normal. Average B. S. for this period, 74 mgm. March 20, condition unchanged; B. S. 78 mgm. Weight 3.00 kilos. Injections of progesterone stopped.

March 21 to 27, condition still good, although appetite poor on occasions. Average B. S. for this period 71 mgm.

March 28, 8:30 a.m. Condition suddenly became much worse; very weak, staggered when compelled to walk; progesterone dosage increased to 20 mgm. daily.

March 29, 8:30 a.m. Condition no better. 3:30 p.m. Convulsions, prostrate, dyspneic respiration. Animal sacrificed. Terminal blood and tissue analyses made: B. S. 45 mgm. per cent. Liver glycogen, 0.11 gram per cent; muscle glycogen, 0.12 gram; cardiac glycogen, 0.27 gram; serum sodium, 120 mE/L.; chloride, 86 mE/L.; potassium, 17 mE/L.; urea, 144 mE/L.

The third male cat showed definite insufficiency symptoms $3\frac{1}{2}$ days following operation. Recovery was effected with progesterone as in the above two cases, and the cat was maintained for 13 days in apparently good health. At this time symptoms of insufficiency recurred, in spite of greatly increased progesterone dosage, and the animal was sacrificed 18 days after operation. Blood-chemical findings revealed conditions indicative of a mild adrenal insufficiency.

It should be added that in all 3 instances above, insufficiency and recovery were well reflected in the frequent blood sugar determinations made throughout the experiments. Moreover, it may be noted that in all cases a definite life-maintaining action on the part of progesterone was demonstrated.

With the hope of discovering whether testosterone might be in part responsible for the extended survival periods described above, 2 male cats were castrated, and, after a period of three weeks, were adrenalectomized. These cats survived during progesterone injection for an average of 21 days. Both animals succumbed, however, during treatment, in spite of heavy progesterone dosage. On the other hand, a definitely beneficial effect of the progesterone was demonstrated, since their average survival period was roughly 4 times that of untreated adrenalectomized cats in this laboratory. It was concluded that castrated male cats may survive adrenalectomy for rather extended periods, although not indefinitely, when injected with progesterone.

Ten female cats were adrenalectomized and experimentation continued with progesterone treatment as above. The average survival period for these animals was 4 days, or approximately the survival expectancy for untreated adrenalectomized cats. In no instance was any ameliorative effect of the progesterone observed, in spite of daily administration of 10 or even 20 mgm. of the preparation. No significant effect of progesterone on the blood sugar was evident at any time and low terminal values were observed in all instances. It was concluded that progesterone has no demonstrable effect on the life-span of adrenalectomized female cats, in marked contrast to its beneficial influence on males. This conclusion had been reached in earlier experiments of a similar nature, and a brief preliminary report was published (1939).

It now appeared that estrone in the blood of the female cats might be responsible for the failure of progesterone treatment in these experiments. Gaunt (1937), working with ferrets, had published results which might be so interpreted. Therefore 3 female cats were ovariectomized, and 3 weeks later when recovery from the initial surgery was complete, both adrenal glands were removed.

One of these animals was maintained in excellent condition for 22 days on a daily dosage of from 4 to 10 mgm. of progesterone. During this period the animal appeared normal in every way, but 4 days after withdrawal of the progesterone it died (26th day after adrenalectomy) with symptoms of acute adrenal insufficiency. Terminal blood and tissue samples were not obtained from this animal due to the rapidity with which the syndrome developed.

The second ovariectomized-adrenalectomized cat refused food from the day of operation and was given 10 cc. of cortico-adrenal extract together with 10 mgm. of progesterone daily for the first 2 post-operative days. Appetite returned and the animal was maintained for 24 days on progesterone alone (total survival, 26 days). It was sacrificed while in good condition, and the blood chemistry showed values well within normal limits. Two relapses during treatment which necessitated increasing the

dosage of progesterone attested to the completeness of adrenal removal as did the autopsy findings.

The third animal reacted in much the same manner as the above, refusing food from the day of operation. Ten cubic centimeters of cortico-adrenal extract plus 10 mgm. of progesterone restored appetite within 2 days and the animal was then maintained on the progesterone alone for a total of 19 days, the dosage being gradually reduced to 5 mgm. per day. Treatment was then stopped and the animal died three days later, exhibiting symptoms of severe adrenal insufficiency. Terminal blood-chemical findings showed the markedly lowered glycemic levels and electrolyte shifts typical of cortico-adrenal hormone depletion.

It was concluded that ovariectomized-adrenalectomized cats may be maintained alive for periods considerably in excess of the usual life-span of similarly operated, untreated animals.

With the assumption that normal luteal secretion coupled with progesterone injection might aid in maintaining life, a further short series of experiments was undertaken. Three pregnant female cats were adrenalectomized and thereafter maintained on progesterone (10 mgm. daily). These animals survived for an average of 13 days. Slight elevation of the blood glucose levels followed the injections, but symptoms of insufficiency occurred precipitately in 2 instances. The third animal was sacrificed 17 days after operation, when marked symptoms of insufficiency appeared in spite of increased progesterone dosage. Blood and tissue chemical values were found to be characteristic of adrenal insufficiency.

It appeared that the luteal factor in the pregnant cat had an ameliorative effect on the development of cortico-adrenal insufficiency, but the effect was slight; even though coupled with injections of progesterone, luteal secretion was incapable of maintaining the life of the adrenalectomized females cats for prolonged periods. The presence of estrone as a factor in precipitating insufficiency symptoms in these animals is suggested.

DISCUSSION. The present experiments demonstrate that progesterone does not maintain blood glucose and liver glycogen in normal, adrenalectomized or hypophysectomized rats. Altogether 30 cases were studied. This is in sharp contrast to the action of cortico-adrenal extract, which was markedly effective in all (20) cases observed. Gaunt, Remington and Edelman (1939) have reported a similar failure of progesterone to affect carbohydrate metabolism in normal (3) rats; they found on the other hand that the liver glycogen levels of fasting ferrets (5) was elevated following progesterone injection.

The life-maintaining effect of progesterone in adrenalectomized (otherwise normal) male cats is demonstrated; thus the onset of symptoms of adrenal insufficiency may be postponed for extended periods (up to 3 weeks) by the luteal hormone. Although undoubted evidence of an ameli-

orative effect was demonstrated in the cat, it is doubtful whether "indefinite" survival of such adrenalectomized animals could be attained by the use of progesterone, even in massive doses. Thus cat 3, although restored with progesterone from symptoms of insufficiency, could not be maintained in health and was sacrificed on the 18th post-operative day in acute insufficiency. Cats 20 and 21 (castrated-adrenalectomized males) also failed to respond favorably to large (10 mgm.) daily injections of the hormone, and developed symptoms of severe insufficiency 18 and 19 days after operation. Further progesterone injections were, moreover, without apparent benefit. One died suddenly and the other was utilized for samples when no improvement in its condition was evident.

On the basis of results obtained with adrenalectomized, non-pregnant females, one is led to the conclusion that progesterone was without beneficial effect in such animals. Since all progesterone-treated male, as well as ovariectomized and pregnant female cats, survived adrenalectomy for rather protracted periods (av. 20 days), it appears that the presence of estrone may explain the non-beneficial effect of progesterone in adrenalectomized (but otherwise normal) female animals.

Aside from theoretical considerations, it is apparent that progesterone cannot be looked upon as a complete substitute for the cortico-adrenal hormone, although recovery from the earlier symptoms of insufficiency and a significantly extended life-span may be demonstrated following adrenalectomy in the cat.

It was noteworthy throughout the study on the cat that an animal's condition was closely related to the glycemic and hepatic glycogen levels encountered. Thus all animals exhibited carbohydrate values within the normal range when in good condition, whether maintained on progesterone or a short course of cortico-adrenal extract to promote post-operative recovery. The ameliorative effects of progesterone on adrenal insufficiency may therefore be possibly attributed to its influence on carbohydrate metabolism.

SUMMARY

Progesterone injection in fasting, adrenalectomized or hypophysectomized rats has no significant influence on glycemic or glycogen levels. The effects of cortico-adrenal extract are in contrast markedly effective under all of the conditions noted.

The life-span of adrenalectomized male, as well as adrenalectomized pregnant or ovariectomized female cats, is considerably lengthened—from 4 to 5 times the usual life expectancy—by progesterone administration. Moreover, recovery from the symptoms of adrenal insufficiency may be effected by progesterone in such animals.

It is doubtful whether survival of the adrenalectomized cat may be indefinitely prolonged by the administration of progesterone.

The condition of adrenalectomized cats, whether treated with progesterone or cortico-adrenal extracts, was related to the glycemic and tissue glycogen levels observed throughout the study.

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COMPARATIVE STUDIES OF THE METABOLISM OF THE BRAIN OF INFANT AND ADULT DOGS[1]

HAROLD E. HIMWICH AND JOSEPH F. FAZEKAS

From the Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, New York

Hughlings Jackson made the suggestion that the nervous system is organized in such a way that the primitive reactions of the older parts are prevented from dominating behavior by inhibiting influences emanating from the higher levels. Thus, removal of some rostral part of the cerebrospinal axis may result in release phenomena. Examples of such release are seen in the decorticate and the decerebrate preparations. Similar phenomena are observed during hypoglycemia (1, 2) and anoxia (3) when the earliest effects reveal depression of the cortex and the basal ganglia and hypothalamus are released. Then the basal ganglia and hypothalamus are depressed while the midbrain becomes dominant. The midbrain is inhibited in turn when the subject exhibits the release phenomenon of decerebration (1, 2, 3).

Hoagland et al. (4) have noted that during marked hypoglycemia the cortical rhythms of the dog disappear before those of the thalamus. By producing cerebral anoxia, Heymans and others (5) have shown that the higher more newly developed parts of the adult brain are less resistant than the older regions. Sugar and Gerard (6) have reported similar results using the electroencephalograph. It has been previously demonstrated that the various parts of the ox brain (7) and rat brain (8) consume oxygen at different rates. The suggestion has, therefore, been made that the order of appearance of the release phenomena of anoxia and hypoglycemia and the alterations in electrical activity may be related to the different metabolic rates of the various parts of the brain. In order to obtain more information on this subject we have determined the oxygen consumption of the various parts of the brain of the adult dog. Additional light may be thrown on this subject by investigating the developing central nervous system. For this reason similar studies were made on the various parts of the brain of growing puppies.

METHOD. The present observations were obtained on minced cerebral tissue of puppy and adult dog placed in the Warburg respirometer and

suspended in a saline solution buffered with phosphate at pH 7.4, with glucose as substrate. The brain of both the young and adult dogs was dissected into the following parts: cortex, thalamus, cerebellum, medulla, midbrain and caudate nucleus. A few observations of the gray matter of the cord of puppies were also made, and in all determinations an effort was made to eliminate the white matter. Puppies from one week to seven weeks of age were studied and the results obtained for each region for each week of post natal life averaged. The parts of the brain were weighed before and after drying at 105°C. and the differences represent the aqueous contents.

RESULTS. The results in the adult brain reveal different rates of metabolism in the various cerebral parts (table 1 and fig. 1). The caudate nucleus possesses the highest oxygen consumption and the medulla the lowest. The cerebral cortex, cerebellum, thalamus and midbrain are

TABLE 1

The oxygen uptake mm³ per 100 mgm. moist tissue per hour at different ages of dogs

PARTS OF THE BRAIN	1ST WEEK		2ND WEEK		3RD WEEK		5-7TH WEEK		ADULT	
	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages
Cortex.........	47	61	26	64	37	68	8	121	25	116
Caudate nucleus	9	73	5	88	6	96	2	139	20	136
Midbrain.......	16	91	14	99	13	111	6	128	10	92
Medulla........	14	96	15	101	19	103	6	85	16	69
Thalamus......	25	76	15	93	14	97	6	124	18	101
Cerebellum.....	14	79	19	85	25	87	9	95	22	107
Spinal cord.....	13	81	7	82	4	93			2	50

intermediate with values of somewhat the same order. The oxygen consumption of these regions of the infant brain one week of age is strikingly different from the adult. In the first place the caudate nucleus and the cortex present values which are definitely less than the corresponding parts of the adult brain. The medulla on the other hand exhibits an oxygen consumption almost twice that of the adult tissue. The few observations of the cord also reveal a higher level in the newborn. The second week of life is characterized by a slight, though significant, increase in the oxygen consumption of practically all parts of the infant central nervous system. The same order as that for the first week of life nevertheless persists with the medulla and cord highest and the cortex lowest. Caudate nucleus and cerebellum possess intermediate. rates similar to the observations of the first week.

During the third week of post natal life the most outstanding phenomenon is that the midbrain changes its relative position and assumes with

the medulla the highest oxygen consumption. The cortex still remains the lowest, while the cerebellum, thalamus, and caudate nucleus are significantly higher than the medulla. From the fifth to the seventh week the oxygen consumption of all parts with the exception of the medulla is definitely higher than the corresponding values for the first week of life. The caudate nucleus possesses by far the highest oxygen consumption at this time. The thalamus and midbrain are second in order. The cortex exhibits an oxygen consumption distinctly higher than either the cere-
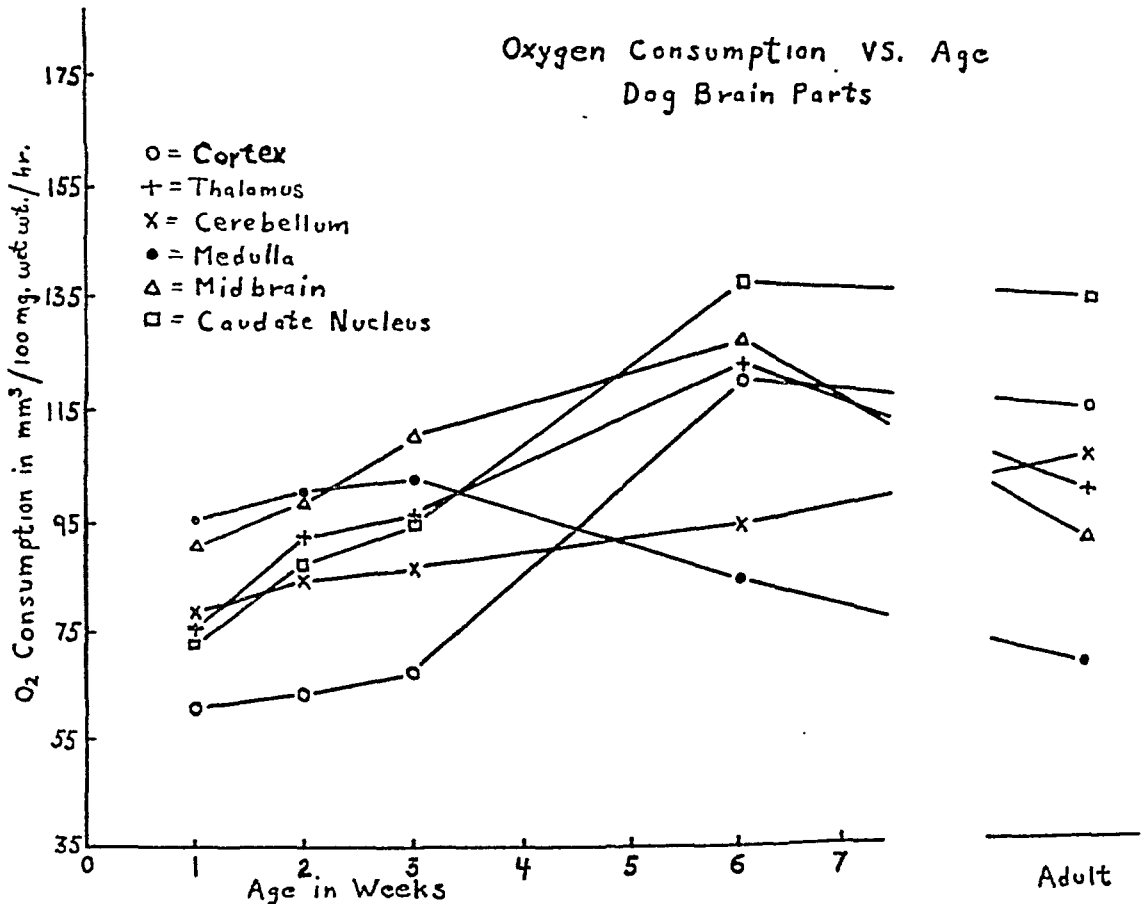
Fig. 1

bellum or the medulla. The latter has at this time an oxygen uptake lower than any of the other parts, lower than the values observed during the first week of life. The observations on the percentages of the aqueous contents of the brain disclose a gradual increase of solids of all the parts studied (table 2). At each age the lower parts are characterized by greater concentrations of solids.

DISCUSSION. In general, these results on dogs are in agreement with previous ones observed on rats (8) insofar that the metabolic rate of the

brain of the newborn is lower than that of the adult. Throughout the first seven weeks of life the caudate nucleus, the cerebral cortex, the thalamus, cerebellum, and medulla exhibit increases in metabolism. A review of table 2 shows a simultaneous rise in solids. These results are not numerous, but they agree with others on rats (9) indicating a greater percentage of dry weight in the brain with increasing age. Whatever determines metabolic rate, whether it is total cellular surface, active respiring surfaces, concentrations of protein or enzymes, probably varies at least partially with total solids (10). In the first week of life the solids of the different regions of the brain are in the same order as their metabolic rates. Thus, the lower parts of the brain contain less water at this time. This relationship, however, does not always hold. Despite a continued decrease in fluid contents the adult dog exhibits a decline of the metabolic rates of some parts of the brain in comparison with that of the puppy in the fifth

TABLE 2

Water contents (per cent) of puppy and adult dog brain

PARTS OF THE BRAIN	1ST WEEK		2ND WEEK		3RD WEEK		5-7TH WEEK		ADULT	
	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages
Cortex.........	27	88.1	14	88.0	5	88.6	5	84.0	6	82.8
Caudate nucleus	2	91.0			1	87.0	2	83.0	3	79.7
Midbrain.......	5	85.0	4	86.5	2	85.0	1	79.0		
Medulla........	5	84.8	4	84.5	1	82.0	3	78.0	1	72.0
Thalamus......	10	86.9	9	86.6	3	87.3	2	82.5	1	70.0
Cerebellum.....	5	87.2	8	85.1	4	84.5	3	82.0	3	83.2

to the seventh week of life. In the adult it is more difficult to separate gray from white matter and it is possible that the apparent decreases in the metabolic rate may be ascribed to the presence of a certain amount of contaminating white matter with its lower metabolism. This applies particularly to the observations on medulla and cord. If the precursor of the white matter in the brain of the newborn either possesses a relatively higher metabolism or represents a smaller percentage of the total tissue, before myelinization takes place, contamination with this material would exert a less important effect on respiration from a quantitative point of view.

The present results have been calculated on a moist basis of 100 mgm. of tissue and, therefore, may approximate the metabolism of the various parts of the brain *in situ* (11). It is, therefore, interesting that a metabolic progression which advances rostrally in the central nervous system is disclosed. The lower parts are most active at birth and as development

continues the wave of metabolism proceeds forward so that the lower portions of the central nervous system are surpassed by the anatomically higher and phylogenetically more recent regions. Other observations in the literature are in accord with this conception. It is generally accepted that early activities of the fetus involve chiefly the lower parts of the neuraxis. Hooker (12) noted that human fetuses, $8\frac{1}{2}$ to 14 weeks old, respond to stimuli only by spinal and medullary reflexes. Even the central nervous system of the newborn operates on the basal ganglion level as evidenced by the sucking and forced grasping reflexes. Cortical functions are practically absent in the newborn. This is in agreement with the low oxygen consumption of the cerebral cortex during this age of life.

Nachmansohn (13) found that choline esterase is present in high concentrations in the spinal cord of sheep fetuses at an early stage of gestation. At this time it is low in the brain centers where it subsequently rises rapidly before birth. Thus Nachmansohn observed increasing activity in the medulla early in gestation, but this activity diminished as myelinization progressed. On the other hand, the caudate nucleus which starts with a lower esterase activity than the medulla finally surpasses it. Thus the mechanisms for the conduction of the nerve impulse as well as for the delivery of energy are developed in the same phylogenetic order and permit the establishment of the functions of the corresponding parts of the central nervous system.

SUMMARY AND CONCLUSIONS

1. With the aid of the Warburg apparatus, determinations were made of the oxygen consumption of various parts of the brain of puppies and adult dogs. The respiratory metabolism of the cerebral tissues was found to be characteristic according to the part of the brain and to the age of the animal.

2. The oxygen consumption of the newborn dog is lower than that of the adult. In the growing puppy during the first seven weeks of life the increase in metabolic rate proceeds rostrally from medulla to caudate nucleus. In the adult brain the respiratory metabolism of the various cerebral parts are higher in the newer phylogenetic layers. These differential rates of metabolism may yield a basis for the order of the symptoms of hypoglycemia and acute anoxia.

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THE INHIBITION OF GASTRIC SECRETION BY THE INTRAVENOUS INJECTION OF CALCIUM SALTS

RHODA GRANT

From the Department of Physiology, McGill University, Montreal, Canada

The essential interest of this problem lies in the hypothesis, for which clinical and physiological evidence exists, that the parathyroid glands in some way, at present not understood, influence the function of the gastric glands.

Babkin, Komarov and Komarov (1, 2) have recently investigated the effects of activated ergosterol and parathyroid hormone on gastric secretion in dogs. In their paper the literature on the subject has been reviewed and, as they have stated, "the data concerning the effect of hyper- and hypocalcemia on the nervous and humoral regulatory mechanisms of the gastric glands are few and contradictory." Their results support the view that the physiological agents which lead to hypercalcemia have an inhibiting effect on gastric secretion—this effect appearing markedly during the nervous phase but only slightly during the chemical.

A change in the level of blood calcium caused by a disturbance of parathyroid function, or by parathormone given therapeutically or to normal animals, or by ergosterol, is accompanied by disturbances in the mechanisms by which these agents maintain normal blood calcium, and any change found in the gastric gland function may be due either to the changes in blood calcium or to the disturbances causing such changes acting directly on the glands. On the other hand, when calcium is injected into the blood the conditions are simplified and the effect of blood calcium *per se* may be studied. But in this case, too, we have to differentiate between injecting calcium as a therapeutic agent to combat a low blood calcium, and injecting it into normal animals, for in the former case we have the complicating factors mentioned above.

The inhibition of gastric secretion, to be described, occurred after the intravenous injection of calcium salts into normal dogs in acute experiments in which the gastric glands were stimulated by histamine or through the vagi with the induction current.

EXPERIMENTAL PROCEDURE. The dogs had access to water but were fasted for 18 hours before the experiment was begun. Throughout the experiment chloralose-urethane anesthesia was maintained and the ani-

mals were kept warm. The following operations were performed: tracheotomy, cannulation of the carotid artery and jugular vein, preparation of the vagi for sectioning later in the experiment, esophagus (in the neck) tied, duodenum tied off, stomach fistulated, urinary bladder cannulated. (In 5 experiments the secretion of the pylorus was collected separately through a cannula in the distal end, the body of the stomach having been separated from it by tying a grooved rubber stopper at the pyloric entrance.) The animal was then placed prone on the supporting frame and during a period of $1\frac{1}{2}$ to 2 hours the resting secretion and excretion were collected. Towards the end of this period the vagi were cut.

When the vagi were stimulating agents for the secretion, rhythmic tetanic stimuli from the inductorium were delivered alternately for $7\frac{1}{2}$ minutes to each nerve. When histamine (dihydrochloride) was used, 0.5 mgm. doses were injected subcutaneously at 15-, 30- or 45-minute intervals, depending on the amount necessary to maintain a constant, suitable rate of secretion. With this aim also in view, the strength of the current when the vagi were being used was regulated.

When the secretion remained constant for two or more 15-minute collection periods, the injection of either 5 per cent calcium lactate or 1.6 per cent calcium chloride was made (in most cases 13 mgm. Ca per kgm. body weight was the dose and cases in which fractions of this were given are indicated in the tables). In a few cases it was possible to give four successive injections with adequate recovery periods between each, without signs of failure of the gastric gland function; on the other hand, in some experiments complete data were obtained only for the first injection, the gastric gland function after this failing to maintain its initial responsiveness.

Blood samples for calcium were taken at infrequent intervals—the first sample after the injection, not before the inhibition had definitely set in. The Kramer-Tisdall (3) method, as modified by Clark and Collip (4), was used.

The blood flow through the stomach was determined by measuring with a stop-watch the time for 5 cc. of blood to flow into a syringe from a cannula placed in the gastro-splenic vein near the spleen, this organ having been cut off from the circulation and the vein clamped distally during the period of collection.

RESULTS. 1. The degree of inhibition, the time after injection at which it was maximum, its duration and after-effects are summarized in table 1. In all, 8 injections of the calcium salts were made in 4 experiments in which the stimulus was through the vagi, and 21 injections in 13 experiments where histamine was used; of the latter, 9 were cases in which the pylorus was excluded.

In all cases inhibition occurred. The average reduction for the 1st

injection, equivalent to 13 mgm. Ca/kgm. body weight, ranged from 22.5 to 97.8 per cent (average, 74 per cent); for the 2nd injection, a fraction of the initial dose, it ranged from 42.0 to 99.2 per cent (average, 76 per cent). The significance ascribed to the fact that the 2nd dose, although as a rule a fraction of the 1st, had as great an effect, will be discussed.

The inhibition was maximum 45 minutes after the injection (variations from 30 to 67 min.) and had ended in 75 minutes (variations from 52 to 105 min.) on an average.   Cases showing the slowest recovery were those, after the 2nd injection, in some of which it was incomplete, in 2 hours. In the cases in which the pylorus was excluded, the average reduction was

TABLE 1

Summary of experiments showing the average reduction in the rate of gastric secretion and the time of maximum inhibition and its duration, following the injection of calcium

STIMULUS	CALCIUM INJECTED PER KGM. BODY WEIGHT	NUMBER OF INJECTION	REDUCTION OF SECRETION (VARIATIONS IN BRACKETS)	INHIBITION, MINUTES AFTER Ca		NUMBER OF EXPERIMENTS
				Maximum	Duration	
	mgm.		*per cent*			
Vagus	13	1st	76.5 (44–96)	45	76	4
Vagus	4.3, 6.5	2nd	83.4 (74.4–92.5)	67	112†	2
Vagus	13	2nd	53.0 (42–64.3)	‡		2
Histamine	13	1st	83.4 (50–97.8)	43	90	6
Histamine	9	1st	40.5	30	60	1
Histamine	9, 9, 6.5, 6.5	2nd	80.9 (42–99.2)	49	110†	4
Histamine	13	3rd	92.2	45	105	1
Histamine*	13	1st and 2nd	61.9 (22.5–87.1)	45	52	4
Histamine*	6.5, 9, 10.4	1st	51.2 (41.2–68)	40	71	3
Histamine*	6.5, 8.6	2nd	65.7 (62.2–69.2)	45	90†	2

* Pyloric secretion excluded.
† Incomplete.
‡ Probably not reached before experiment ended.

less and the period of inhibition shorter than in the cases in which the total secretion was collected.

Following the period of inhibition an increase over the control volume of secretion was observed in 13 cases which were carried on over sufficiently long periods.   This increase amounted to 47 per cent (variations 5 per cent to 103 per cent) and occurred $1\frac{3}{4}$ hours (variations $\frac{3}{4}$ to 3 hrs.) after the injection.

2. The comparative effects of sodium lactate and calcium lactate are shown in figure 1, representing one of 4 experiments, of which this one, in which it was possible to give 3 or 4 successive injections, provided us with the best conditions for a comparison.   The sodium lactate caused no inhibition in rate of secretion but rather the reverse.

3. With respect to the mechanism of inhibition, an osmotic pressure change was eliminated as an important factor by using solutions isotonic with blood. In addition the changes in urinary excretion were only very slight; and the freezing-point of the blood, determined at the critical periods of a typical experiment, showed no change which could be considered significant (fig. 2). That circulatory changes were not important causal factors was the conclusion from results of experiments in which the maximum inhibition of secretion was seen long after the blood pressure (table 2) and blood flow through the stomach (table 3) had returned to normal. The experiment given in table 3 is one of two in which the blood flow through the stomach was measured. The gastric secretion was not registered.
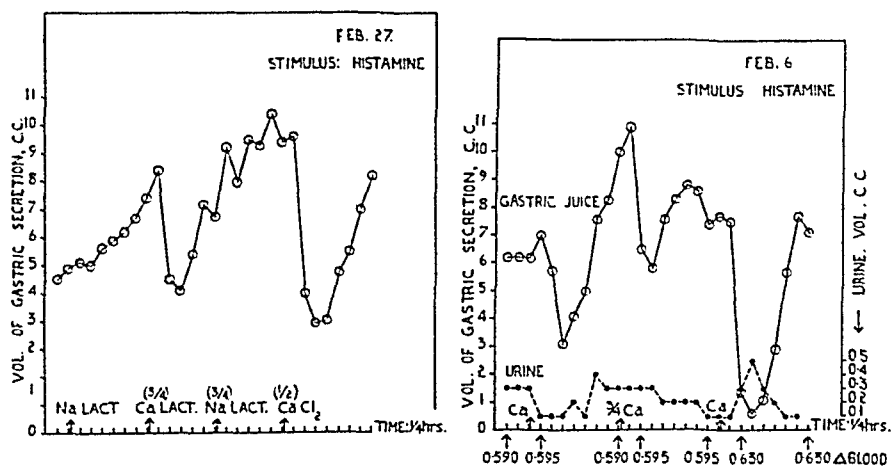
Fig. 1. Left. The comparative effects of calcium lactate, calcium chloride and sodium lactate on gastric secretory rate.

Fig. 2. Right. Effect of calcium lactate on volume of gastric secretion and on osmotic pressure of blood.

4. The blood calcium results (table 4) are taken from all experiments and give a general picture of the changing level. Figures taken from an experiment of Smith and Sternberger (5) are complementary to ours and make it appear probable that the maximum calcium of 16 to 17 mgm. per cent was reached a few minutes after the injection.

DISCUSSION. In these experiments the calcium salts inhibited both the nervous and the chemical type of secretion. From this it would be inferred that the effect is not a specific interference with the initiation or transmission of the nervous impulse, as appeared to be the case in the experiments of Babkin, Komarov and Komarov (1), especially those with ergosterol. However, in their experiments a much more profound disturbance involving the obscure processes controlling the blood calcium level must exist, in contrast to the conditions in mine where we are dealing simply with an excess of calcium, probably in the ionized form, which

merely may have to be excreted by one route or another, or stored.   It is possible that the difference between the ergosterol and the parathormone effects found by Babkin *et al.* may be due to a difference in the action these agents have on the balance of the calcium fractions, because as was shown by Mr. Schiffrin in this laboratory (see Babkin, 2) parathormone produces a more marked inhibition of histamine secretion than does ergosterol.

TABLE 2

Blood pressure changes compared to changes in the rate of gastric secretion after intravenous injection of 5 per cent calcium lactate or normal saline or 1.2 per cent sodium lactate

TIME	MARCH 29				APRIL 2*		APRIL 5		APRIL 9†	
	1st injection		2nd injection		B.P.	Vol.	B.P.	Vol.	B.P.	Vol.
	B.P.	Vol.	B.P.	Vol.						
Before 13 mgm. Ca	112	5.8	104	12.0	58	7.2	100	6.4	96	3.4
2–3 min. after 13 mgm. Ca	150		200		72+		122+		136	
15 min. after 13 mgm. Ca	110	4.4	100	5.3			112	2.3	98	3.6
30 min. after 13 mgm. Ca	110	5.3			54	2.6	104	1.4	96	2.0
45 min. after 13 mgm. Ca			94	1.6			106	1.1	84	2.7
60 min. after 13 mgm. Ca					56	7.3			86	2.8
75 min. after 13 mgm. Ca			76	13.3						
Before 0.9 per cent NaCl	100	5.5	126	9.6			120	6.2		
2–3 min. after 0.9 per cent NaCl	112		112				118			
15 min. after 0.9 per cent NaCl	112	5.4	112	10.0			118	6.3		
30 min. after 0.9 per cent NaCl							100	8.1		
45 min. after 0.9 per cent NaCl	112	5.5	104	10.9			96	7.7		
Before Na lactate									126	6.9
2–3 min. after Na lactate									134	
15–30 min. after Na lactate									134	6.3
45–60 min. after Na lactate									112	5.0
75–90 min. after Na lactate									112	6.8

* Splanchnics cut.
† 6.5 mgm. Ca.

In solving the question of whether or not parathormone or ergosterol inhibits more or less specifically the nervous or vagal type of secretion, these experiments can be of little value; however, they should be valuable in giving information on the effect *per se* of hypercalcemia.   It appears that this has no specific action—both types of secretion being inhibited.

The relation between inhibition and hypercalcemia can be seen by com-

paring the reduction in secretion caused by the full amount (13 mgm. per kgm.) of calcium with that caused by fractions of the dose. In many experiments, too, the 2nd and 3rd doses of calcium, injected when the volume of secretion had returned to normal after the 1st, caused as great,

TABLE 3

Changes in blood flow through the stomach and systemic blood pressure changes after injection of calcium (13 mgm./kgm.)

TIME	BLOOD FLOW	BLOOD PRESSURE	REMARKS
	cc./min.	mm.	
Before calcium...........	12.80	136	
3 min. after calcium......	30.00	164	
20 min. after calcium.....	12.50	136	
30 min. after calcium.....	12.50	126	
45 min. after calcium.....			Time of maximum inhibition
60 min. after calcium.....	9.12	110	
90 min. after calcium.....	9.08	86	Inhibition ended
98 min. after calcium.....	6.60	74	B.P. lowered artificially
100 min. after calcium....	6.80	86	
110 min. after calcium....	12.00	98	B.P. raised by calcium injection

TABLE 4

Blood calcium changes after the 1st injection of calcium (13 mgm./kgm.) compared with observations of Smith and Sternberger

TIME	BLOOD CALCIUM		NUMBER OF SAMPLES	BLOOD CALCIUM FIGURES FROM EXPERIMENT OF SMITH AND STERNBERGER (11.3 MGM. Ca/KGM. INJECTED)
	Average	Variations		
	mgm. per cent	mgm. per cent		mgm. per cent
Before calcium..........	10.1	9.3–11.0	8	9.9
4 min. after calcium.....				15.4
15 min. after calcium....	16.0		1	13.4
30 min. after calcium....	13.9	13.4–14.4	3	12.5
45 min. after calcium....	12.7	11.9–13.5	4	
60 min. after calcium....	13.1		1	
90 min. after calcium*...	12.8		1	11.0
105 min. after calcium...	12.7		1	
120 min. after calcium...	11.9	11.8–12.0	2	
135 min. after calcium...	11.0		1	
5 hrs. after calcium......				9.5

* Marks the end of the period of inhibition of gastric secretory rate.

or even greater reduction in the rate of secretion than the initial one, even though they were smaller. This I associated with the fact that the blood calcium was restored to normal more slowly than the volume of secretion (tables 1 and 4); thus the 2nd and 3rd injections were made into blood con-

taining calcium 2 to 3 mgm. above the normal level, which would appear to be the threshold. In apparent contradiction to the inter-relationship of these two factors, we have the events of the 15-minute period immediately following the injection when the blood calcium is at its maximum, but still no inhibition occurs. This could be perhaps partially explained by the sharp transitory rise in blood pressure which takes place in this period, accompanied by the increase in blood flow through the stomach; these circulatory changes, by increasing the head of pressure, may be obscuring a direct action of calcium on the gastric glands immediately after the injection.

A point which may prove to be of some significance but for which, at present, I have no explanation to offer, is the difference in the degree of inhibition between the group in which the secretion from the whole stomach was being measured and that in which the pylorus was excluded; in the latter the average reduction was less and the period of inhibition shorter.

CONCLUSIONS

In acute experiments on dogs the injection of calcium lactate or calcium chloride inhibits both the nervous and the chemical type of secretion.

The inhibitory agent appears to be the calcium radicle, since equivalent amounts of lactate as the sodium salt failed to have the inhibitory effect of either of the calcium salts. The degree of inhibition depends to some extent on the degree of hypercalcemia.

The hypercalcemic effect, under the conditions of these experiments, is not specific for nervous or chemical responses of the gastric glands, and in this differs from the action of parathormone and ergosterol in the chronic experiments of Babkin and his co-workers.

The mechanism for the inhibition has not been found. Osmotic pressure changes in the blood were not caused by the solutions and circulatory factors, i.e., general blood pressure and blood flow through the stomach could not be correlated with the changes in secretion rate caused by the calcium.

I am indebted to Prof. B. P. Babkin, who suggested, and maintained an unfailing interest in, this problem.

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THE RELATION OF CALCIUM CONTENT TO ACIDITY AND BUFFER VALUE OF GASTRIC SECRETIONS

RHODA GRANT

From the Department of Physiology, McGill University, Montreal, Canada

Variations in the calcium content of gastric juice were observed in an investigation (1) in which the inhibitory effect of hypercalcemia on gastric secretion was being studied. The variations bore an inverse relationship to the acidity of the secretion. Such a relationship has been observed recently, independently of us, by Kirsner and Bryant (2). Apart from this, isolated observations of calcium changes made in the course of studies of the composition of gastric juice have been reported. Some of these lend support to our results, without having received particular attention from their authors.

A systematic study of the relations between the calcium content and the acidity of gastric juice in dogs under various conditions of secretion are reported in this paper.

LITERATURE. Rosemann (3) reported the calcium content of gastric juice in the dog after sham-feeding to be 0.22 to 0.07 mgm. per 100 cc. Gamble and McIver (4) in a study of the acid-base composition of juice from Heidenhain pouches in cats during fasting and at intervals after food reported 4.1 mgm. in the fasting state, 10.6 mgm. 2 to 4 hours after food, and 5.7 mgm. 8 to 12 hours after food. Rudd (5) in two papers dealing with the composition of gastric juice in man found 4.1 to 8.6 mgm. per cent. This author observed that calcium was present in minimal amounts some time after the injection of histamine. Bonne, Hartz et al. (6) found 3.4 to 4.4 mgm. per cent in the secretions obtained with alcohol or caffeine tests in different racial types. Austin and Matthews (7) found an average of 6.08 mgm. per cent in 11 normal dogs, and of 6.65 mgm. per cent in 12 made hypercalcemic with parathyroid extract. The experiments of Kirsner and Bryant (2) were performed on 70 patients— 5 with the Ewald test meal, 65 with histamine—and on 5 dogs, using either fasting or fundic pouch secretions. Values for the human cases ranged from 0.85 to 7.00 mgm. per cent and in general the higher the value the higher the pH found. It was also noted that the calcium was higher in fasting or Ewald test-meal secretions than in those after histamine, but that patients with achlorhydria were exceptions to low values with his-

tamine. In the fasting secretion of a pregnant dog the calcium ranged from 11.07 to 14.8 mgm. per cent, and in the histamine secretion was 11.07. The values for fundic pouch secretions of 4 dogs were much lower, i.e., 1.10 to 3.30.

The calcium variations to be described were found in the dog under acute conditions. These variations were accompanied by changes in other factors; the acidity bore an inverse, and the buffer value a direct, relationship.

METHODS. Details of the experimental procedure have been described in the previous paper (1). Each 15-minute sample of secretion, after having been measured, was filtered through ashless filter paper and placed
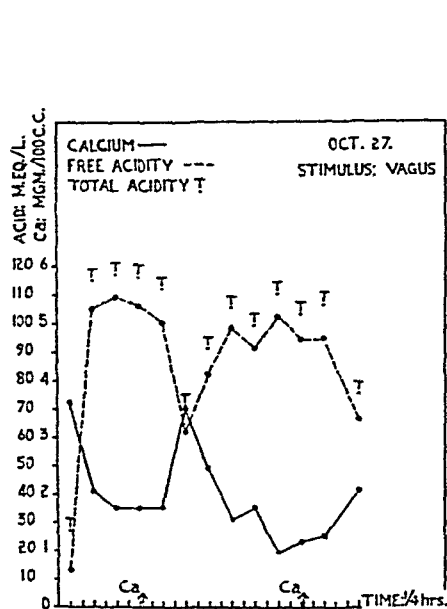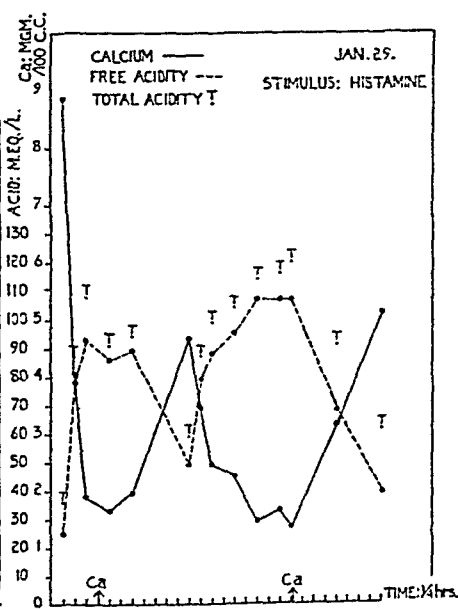
Figs. 1 and 2. Reciprocal relationship between acidity and calcium concentration of gastric juice.

in the cold until the next morning, when it was analysed. Total and free acidity were determined with Töpfer's and phenolphthalein indicators; total chloride by the modified Volhard-Harvey method (8); pepsin by Mett's method as modified by Nierenstein and Schiff (9); and calcium by treating the filtered secretion as an acid extract of ashed material as in the Tisdall and Kramer method for calcium in urine and other biological materials (10). Values obtained by this method were the same as those found after ashing the secretion. In pyloric secretions where the viscosity was very high the material was ashed.

RESULTS. The most obvious result of these experiments was the reciprocal relationship between the calcium concentration and the acidity of the secretion. Evidence that a high acidity is accompanied by a low

calcium and *vice versa* was seen under three different conditions: *a.* Following the intravenous injection of calcium salts. Figures 1 and 2 are typical examples of a total of 29 injections into 17 animals. The relationship held for secretions of both the histamine and the vagus type. *b.* During the control period before the calcium injection (table 1). It was a practical experience that the initial levels of acidity in any animal gave a general indication of the inital amount of calcium to be expected—whether high or low. *c.* The third type of evidence was free of certain criticisms which might be made of the first two—viz., of *a*, that the conditions of an artificial hypercalcemia are not normal, and of *b*, that individual differences from one experiment to another may lead to erroneous conclusions. In the third type vagal and histamine stimuli were given alternately in the same experiment (fig. 3). To obviate the criticism that depletion of the calcium stores might have caused the lower values in the second or histamine period which occurred in the first experiments, the order of the

TABLE 1

Comparison of initial levels of acidity and calcium concentration

ACIDITY	CALCIUM CONCENTRATION		NUMBER OF EXPERIMENTS
	Average	Variations	
m.eq./l.	*mgm./100 cc.*	*mgm./100 cc.*	
25– 75	6.67	3.73–8.70	4
76–100	4.05	1.47–6.40	7
101–125	2.97	0.55–4.89	9
126–150	1.91	0.46–4.19	4

stimuli was reversed but the vagal stimulus following the histamine had little if any effect. The other course open was to try to use the vagus in a 3rd period of stimulation after the hypothetical exhaustion of the calcium stores. This proved to be successful in spite of the fact that the final period of vagal stimulation followed a histamine period. This was attributed to the unusually small amount of histamine necessary to start and maintain a good volume of secretion, after the initial vagal period, compared to that necessary to yield a corresponding volume when the histamine was used alone. Thus the amount of histamine was not great enough under the former conditions to abolish the effect of the second vagal stimulus which followed it. In this experiment (fig. 3) we switch from a vagal secretion of low acidity and high calcium to that characteristic of histamine with high acidity and low calcium. In the final period a reversal to the characteristic vagal type is occurring, which shows that the low calcium values in the histamine period were not due to exhaustion of the calcium. This experiment has been repeated 5 times in cats with the same results.

Two exceptions to the reciprocal relationship between calcium and acidity may be of importance. In a number of cases in which free acid was absent in the initial samples the calcium was not at its maximum height, as might be expected, but rose to a higher level on the appearance of free acid. In the other exceptional case, the unusual combination of high levels of acidity and high "buffer values" (cf. below) in some samples were accompanied by very high calcium values.

The possibility that the changes in calcium concentration were simply reflections of the volume changes, with which it is generally accepted the acidity varies, is not supported by the finding that in the 21 cases in which
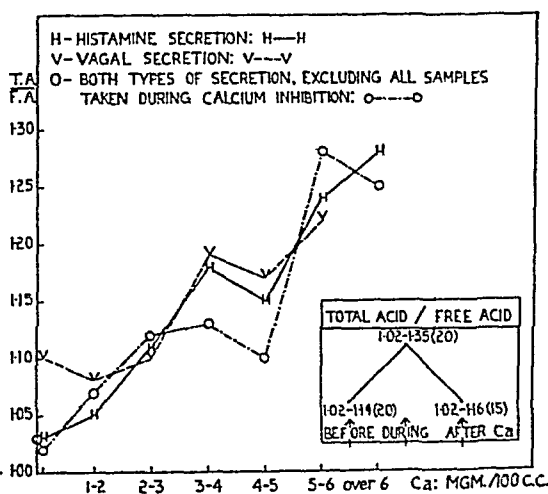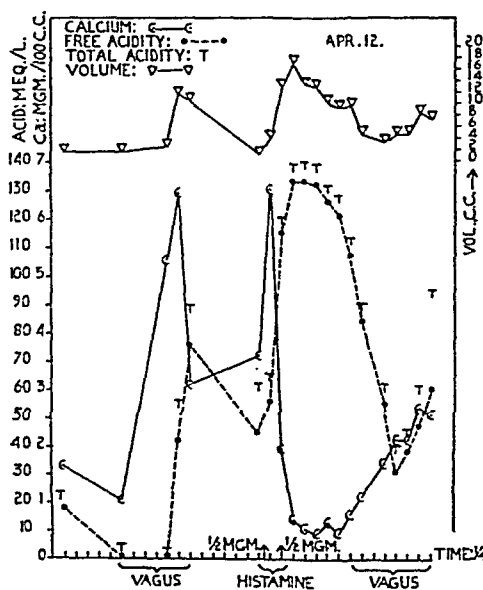
Fig. 3. Left. Changes in calcium and acidity caused by alternate vagal and histamine stimuli.

Fig. 4. Right. Relationship between calcium and ratio $\dfrac{\text{Total acid}}{\text{Free acid}}$.

Inset curve: Ratios before, during and after calcium—showing range of values and (in brackets) number of cases.

the data were complete for changes after calcium injection—i.e., those in which the initial volume was restored after the calcium effect had worn off—the acidity was lower and the calcium concentration higher than the control levels when the volume had already been restored after the period of maximum inhibition. Also when vagal and histamine stimuli were used alternately, in spite of parallel volumes in some periods, the characteristic differences in acidity and calcium for the two types of secretion were seen (fig. 3).

The variations in calcium output in these experiments lend support to the belief that the volume changes are not entirely responsible for the

changes in calcium concentration. When the decrease in output below the initial level occurring during the period of maximum inhibition of secretion was balanced against the subsequent increase in output, the balance was positive by amounts varying from +2 per cent to +204 per cent in 14 cases; in 7, however, the loss which occurred was not made up—the deficit amounting to from −8 per cent to −57 per cent. The calcium output increased when the vagal stimulus replaced the histamine.

TABLE 2

Total acidity/Free acidity ratios and corresponding calcium values in vagal and his-tamine types of secretion

	VAGAL SECRETION		HISTAMINE SECRETION	
	Ratio	Calcium	Ratio	Calcium
	m.eq./l.	*mgm./100 cc.*	*m.eq./l.*	*mgm./100 cc.*
March 1 (Dog)				
Average	1.09	4.65	1.02	0.43
Variations..................	1.06–1.23	1.83–13.20	1.02–1.03	0.00–1.07
Number of cases.............	6	6	7	7
March 8 (Cat)				
Average....................	1.08		1.03	
Variations..................	1.07–1.10		1.03–1.05	
Number of cases.............	5		7	
April 12 (Dog)				
Average....................	1.20	3.83	1.03	0.79
Variations..................	1.08–1.55	1.68–6.47	1.03–1.04	0.45–1.98
Number of cases.............	13	13	6	6

TABLE 3

SECRETION FROM	CALCIUM		TOTAL NUMBER OF CASES	SPREAD OF CALCIUM VALUES		
	Average	Variations		0–5 mgm. per 100 cc.	5.1–10 mgm. per 100 cc.	Over 10 mgm. per 100 cc.
	mgm./100 cc.	*mgm./100 cc.*				
Pyloric part............	9.8	1.9–23.1	30	1	20	9
Fundus + corpus.......	1.7	0 – 4.6	25	25	0	0

An increase in the ratio Total acidity/Free acidity accompanied the increase in calcium concentration, whether this was due to injection of calcium or to replacement of histamine by vagal stimulus. This relationship also held when the control values of different experiments were compared. (Fig. 4 and table 2.)

The calcium concentration in pyloric secretion (always very viscous) was higher than in that collected over the same period from the rest of the stomach. In table 3 some comparative figures are listed.

DISCUSSION. Independently of volume changes a high calcium and low acidity appear in gastric secretion after the intravenous injection of calcium and during vagal stimulation in contrast to the lower calcium and higher acidity occurring before the calcium injection and during histamine stimulation. This behaviour is most convincing when the changes are caused in a physiological way by stimulating alternately the cells responding to histamine and to the vagus. The association between these two factors is at present obscure. If the source of the calcium is some component of vagal secretion, as appears probable, the effect of acid coming in contact with this in the early stages of secretion and its disappearance finally from the acid secretion are problems at present being considered.

The increase in the ratio Total acidity/Free acidity constantly appears with an increase in calcium. If, as Rudd (5) concludes from his experiments on the effect of mucoprotein on the titration of HCl to the phenolphthalein end-point, when the titration is carried on from the Töpfer's end-point to that of phenolphthalein, the mucoprotein dissolves in the added alkali and the difference in the two readings is due mainly to the combination of NaOH and mucoprotein—then the differences in the ratios in our experiments are a measure of the amount of mucoprotein or other buffer present. The ratio then is an index of the buffer value. There appears to be an association between this factor and the amount of calcium occurring in the samples of secretion.

SUMMARY

Variations in the calcium concentration of gastric secretions of both the nervous and the chemical type obtained under acute conditions from dogs bear a reciprocal relationship to the acidity of the secretions.

This reciprocal relationship was found when the calcium and acidity changes were either spontaneous or induced; viz. 1, when comparing the initial levels of calcium and acidity in 24 animals; 2, in single experiments when vagal and histamine stimuli were used alternately to produce their characteristic secretions of low or high acidity respectively; 3, when the factors were changed by intravenous injection of calcium salts.

The relationship is not dependent on volume changes. The output of calcium increased after the period of inhibition caused by calcium injection and during vagal stimulation.

The ratio Total acidity/Free acidity which is interpreted here as an index of the buffer value varies directly with the calcium changes brought about in the ways mentioned above.

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THE DISTRIBUTION OF INJECTED RADIOACTIVE POTASSIUM IN RATS

T. R. NOONAN, W. O. FENN AND LORRAINE HAEGE

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York

The predominantly intracellular distribution of potassium is well established, but the mechanism by which this intracellular concentration is maintained is not well understood. With the development of an artificially radioactive isotope of potassium K^{42}, a new method of attack upon the problems associated with the metabolism of this element has become available. We have employed this new technique to measure the magnitude and rate of total uptake of injected potassium by various types of tissues, thus gaining an insight into the relative permeabilities of different cells to potassium.

Two previous studies on the distribution of K^{42} after injection have been published. Greenberg et al. (1938) and Joseph, Cohn and Greenberg (1939) used albino rats and determined the rate of uptake of the labelled atoms per gram of tissue for muscles, liver, kidney and blood (but not plasma) after intraperitoneal or oral administration. Hahn, Hevesy and Rebbe (1939) have described the distribution of K^{42} in a single rabbit 24 hours after subcutaneous injection and also in the plasma, muscle and bone of a few frogs. In both of these investigations, however, adequate interpretation of the results is impossible because of lack of analyses for the total potassium of the tissues under consideration although Hahn et al. have made use of certain assumed potassium values in the interpretation of their results.

In our work we have determined total potassium as well as radioactivity of tissue samples taken at varying times after the injection of K^{42}. This is necessary in order that the results may be interpreted properly, for the radioactivity of any tissue depends first of all upon the amount of potassium which it contains in a form available for exchange with its radioactive isotope. It has become evident that injected potassium passes into all tissues of the body, but at varying rates which are high in liver, heart, kidney and lung, intermediate in muscle and skin, and low in testes, erythrocytes and brain. Approximate equilibrium is secured about six hours after injection, but red cells and brain are not in complete equilibrium

even after 18 hours.   In general our results are in agreement with data published by Joseph, Cohn and Greenberg but are in conflict with the findings of Hahn, Hevesy and Rebbe that very little exchange of potassium between plasma and tissue cells occurs.

METHODS.   Albino rats were used as the experimental animals, usually without previous fasting or other preliminary treatment.

The activated potassium was prepared by deuteron bombardment of samples of chemically pure potassium chloride weighing 100 to 150 mgm. In order to free the sample from possible contamination with radioactive sodium, the activated potassium salt was dissolved in a minimal volume of water and precipitated as the perchlorate in a Gooch filter crucible by the addition of saturated ammonium perchlorate solution.   The potassium perchlorate precipitate was washed and converted to the chloride by heating in the presence of a trace of manganese dioxide.   After being weighed the potassium chloride was dissolved in the crucible, filtered by suction and made up to a volume of 10 ml.   The loss of weight of the crucible was determined and was assumed to represent the potassium chloride in the solution.

The radioactive solution, containing usually about 1 per cent potassium chloride, was injected by one of several routes.   After varying intervals of time, the animals were sacrificed by cutting the throat, and the blood was collected by holding the wound over a beaker.   Weighed samples of tissues (0.5 to 1.0 gram) were dissolved in concentrated nitric acid in tared weighing bottles or graduated centrifuge tubes.   From the volume of the digest (either read directly on the graduate or calculated from the weight of the digest and its measured specific gravity) suitable aliquots were taken both for determination of the radioactivity by a Geiger-Müller counter as modified by Bale, Haven and LeFevre (1939) and for total potassium content by the Shohl and Bennett method with minor modifications (Fenn and Cobb, 1936).   A suitable dilution of the injected K^{42} solution was used as a standard in measuring the radioactivity of the tissue samples.   After deduction of the background count (determined with 3 ml. of distilled water around the ionizing chamber in place of 3 ml. of digest) the K^{42} counts were plotted against time on "semi-log" paper. Linear interpolations between points on this graph served for the determination of the simultaneous standard count for each sample count.   The sample counts were then expressed as a percentage of the simultaneous K^{42} standard count.   This procedure corrects for the decay of the radioactive isotope as well as for any changes in the efficiency of the counter. Standard count and background counts were made approximately every two hours while the samples were being counted.   The background counted was usually about 2 per minute and the standard and samples about 10 to 100 per minute.   Most solutions were counted for 15 minutes, or longer if the count was very low.

The following calculations have been made from the data:

$$\text{Activity} = \frac{\text{counts found per kgm. wet weight of sample}}{\text{total counts injected per kgm. body weight}}$$

$$\text{Potassium activity} = \frac{\text{activity} \times 100}{\text{millimols of potassium per kgm. of wet tissue}}$$

$$\text{Relative potassium activity} = \frac{\text{potassium activity of tissue}}{\text{potassium activity of plasma}}$$

$$\text{Exchanged potassium in mM per kgm. body weight} = \frac{100}{\text{potassium activity of plasma}}$$

When mixing is complete the exchanged potassium is the same as the total potassium in the body. The activity has a value of 1.0 when the injected potassium is evenly distributed throughout all the body weight. The potassium activity has been multiplied by 100 merely for convenience. It may be noted, however, that this is not far from the number of millimols of K per kilogram body weight of an average rat. If the correct figure were known in each case and were used in place of 100 then the potassium activity so calculated would have a value of 1.0 when the injected potassium was evenly distributed throughout all the body potassium. The relative potassium activity ordinarily has a value of 1.0 when exchange between a given tissue and the plasma is on the average complete. There is no way of knowing of course whether all parts of the tissue sample were equal in their potassium activity. Some parts of the sample may have been higher and others lower than the plasma.

RESULTS. The results of a single complete experiment are given in detail in table 1. The table is self-explanatory. It will be seen that only 85 per cent of the injected counts were recovered. The discrepancy is presumably due to various small losses during the experiment. The loss in the excretion was 9.2 per cent which includes both urine samples and probably feces. The figure 1.30 for the potassium activity of the urine is based on analysis of the second urine sample and presumably represents the average potassium activity of the plasma during the last 13 hour collection period. The K activity of the plasma at the end of that time was somewhat lower (1.045).

In table 2 are listed most of the actual figures obtained in our experiments on rats. Values are given for the activity (act) and the total potassium content (K) in m.eq. per kgm. wet weight of the various tissues. From these two values, the potassium activity may be calculated. The experiments are arranged in order of duration and are grouped according to the method of administering the radioactive isotope. Dosage, calculated total potassium, and average potassium contents for the various tissues are also included in table 2.

In eight experiments including that shown in table 1 an attempt was made to recover from the rat all the counts injected. This involved

TABLE 1

Protocol of typical experiment—18 hour rat, no. 26

4/16/40 3:30 p.m.—192 gram male rat from stock, injected subcutaneously with 4 ml. 1.47 per cent radioactive KCl solution and placed in metabolism cage.

4/17/40 8:30 p.m.—Urine collected, sample A. 9:30 a.m.—Urine collected, sample B. Rat killed by cutting throat, blood collected with oxalate by holding wound over beaker, tissue samples dissected, weighed and dissolved in nitric acid.

TISSUE	WEIGHT OF SAMPLE	COUNTS PER MIN. COR-RECTED	ACTIVITY	POTAS-SIUM	POTAS-SIUM ACTIVITY	RELATIVE POTAS-SIUM ACTIVITY	COUNTS IN WHOLE SAMPLE, PER CENT OF TOTAL
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	grams			*mM/kgm.*			
Plasma............	1.65	834	0.00802	7.66	1.045	1.00	0.07
Liver..............	5.4	11,150	0.107	102.6	1.045	1.00	3.01
Kidney............	1.325	9,660	0.093	79.1	1.17	1.12	0.64
Heart..............	0.616	9,900	0.095	83.8	1.13	1.08	0.31
Gastro-int..........	12.4	7,500	0.072	62.8	1.15	1.10	4.65
M. gastrocnemius...	1.100	13,750	0.132	116.2	1.14	1.09	0.76
Red cells..........	2.2	8,380	0.081	97.2	0.83	0.79	0.92
Brain..............	1.215	5,160	0.050	106.0	0.47	0.45	0.31
Testes.............	2.018	8,170	0.084	94.0	0.89	0.85	0.88
Skin...............	45.9	3,880	0.037	52.8	0.70	0.67	8.90
Diaphragm.........	0.443	11,015	0.105	86.9	1.21	1.16	0.24
Lung..............	1.104	9,350	0.089	83.8	1.06	1.01	.52
Residue............	111.0	9,850	0.095	82.4	1.15	1.10	54.67
Excreta............					1.30	1.24	9.20
Total..............	186.37						85.08

The weights given in column 2 represent the whole organ or tissue except in the case of muscle where only one gastrocnemius was used. Some blood was lost in cutting the throat. Column 3 is expressed in terms of the counts per min. given by the simultaneous standard solution. The standard used was the injected solution diluted 500 times. Column 3 is the counts per 5 min. in a 3 ml. sample of the nitric acid digest $\times$ (total vol. of digest $\div$ wt. of sample) $\div$ simultaneous standard count $\times$ 1000. The standard used was the injected solution diluted 500 times. Column 4 is column 3 $\div$ 10.41 $\times$ 10^6, the number of counts injected per kgm. body wt. The number of counts injected was 4 ml. $\times$ 500 (dilution) $\times$ 1000 (for convenience) = 2×10^6. Column 5 is calculated from the analysis of the nitric acid digest. Column 6 is col. 4 $\div$ col. 5 $\times$ 100. Column 7 is col. 6 $\div$ 1.045. Column 8 is col. 3 $\times$ col. 2 $\div$ $2 \times 10^6 \times 100$.

The exchanged $K = \dfrac{100}{1.045} = 96$ millimols K per kgm. Adding up all the K found in the samples and the residue gives a total of 74.3 millimols per kgm. The discrepancy is partly due to loss of K in the excreta which was not accounted for.

sampling all the tissues as usual and in addition dissolving everything remaining after the dissection (carcass) and analyzing it as a whole for

TABLE 2

Activity (Act.) and potassium content (K) of various tissues

RAT NO.	ROUTE	TIME	WEIGHT	CALC. TOTAL K	DOSE OF K	PLASMA		LIVER		KIDNEY		HEART		GI		MUSCLE		ERYTHROCYTES		BRAIN		SKIN		TESTES		DIAPHRAGM		LUNG	
		hours	gm.	m.eq./kgm.	m.eq./kgm.	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K
1	IP	0.5	151	59	2.8	0.151	9.1	3.04	92	1.57	40	1.99	91	2.49	79	0.09	116	0.12	80	0.05	103	0.48	48						
2	IP	0.5	153	55	(2.3)	0.153	8.6	2.83	89	0.87	76	2.04	80	0.81	62	0.09	112	0.18	103	0.06	96	0.20	38					0.84	63
3	IP	1.0	237	80	(2.2)	0.133	11.8	3.27	109	1.44	80	1.61	93	1.99	60	0.02	121					0.20	44						
4	IP	1.0	202	66	(1.7)	0.141	9.6	5.96	96	1.58	89	1.98	95			0.66	94	0.18	78	0.14	*92								
5	IP	1.0	316	46	1.1	0.104	4.9	4.10	(91)							0.23	118	0.28	99	0.08	(102)								
6	IP	1.0	215	87	3.2	0.070	6.4	0.45	95	0.99	80	1.25	89			0.13	114	0.08	83	0.03	113							0.60	86
7	IP	1.0	240	63	(2.2)	0.151	9.8									0.02	129	0.15	90										
8	IP	1.0	318	66	2.0	0.178	11.7	3.20	91							1.35	112	0.18	88										
9	IP	1.6	230		1.0	0.184		4.07	91	1.93	75	2.07	86			1.66	117			0.11	108								
10	IP	2.0	242		1.0	0.145		3.37	(91)			1.96	(84)			1.10	(113)												
11	IP	2.0	200	37	2.7	0.183	5.6	7.50	99	2.32	53	3.54	89			1.88	117	0.62	97	0.20	105	0.50	(43)	0.61	95	5.64	86	2.06	71
12	IP	3.0	252	83	2.8	0.058	7.6	2.32	101	1.41	82	1.16	81			1.44	76	0.40	86			0.58	36					0.91	(81)
13	IP	3.0	312	46	0.9	0.187	9.7	2.93	(91)			2.18	82	1.40	50	1.96	114												
14	IP	5.0	291	105	0.7	0.090	9.6	1.76	92	1.28	70	1.39	83	1.51	88	0.96	120	0.50	96	0.28	110			0.54	93	1.76	100	1.41	83
15	IP	5.0	289		0.7	0.114		1.23	75	0.86	66	1.17	87	1.67	102	1.47	120	0.34	76	0.25	106					1.58	100	1.08	78
16	IP	6.5	184	64	2.8	0.166	11.0	1.75	109	0.74	64	1.51	(84)	1.31	83	2.32	122	0.55	97	0.28	109			0.58	76				
17	IP	10.0	195	98	(3.0)	0.110	11.0	1.01	90	1.07	73	0.88	75	1.13	74	1.36	123	0.55	101	0.33	94	0.49	39	0.12	96				
18	ST	1.0	208	83	2.8	0.146	12.6	3.50	107	1.62	83	1.68	82	3.25	80	0.55	104	0.15	84	0.08	110	0.33	40	0.21	96			1.54	70
19	ST	2.0	193	67	3.3	0.116	8.2	2.63	101	1.76	79	2.05	87	2.72	80	1.08	117	0.21	100	0.15	98	0.56	45						
20	SC	1.0	246	69	0.8	0.173	12.0	1.28	84	1.65	79	1.89	91	0.86	65	0.66	113	0.17	95							1.45	92	1.13	80
21	SC	1.0	208	71	0.3	0.12	8.4	1.29	102	2.12	(75)	2.20	(84)	1.24	90	0.78	115	0.10	96							1.45	110	1.04	98
22	SC	1.0	230	38	2.3	0.200	6.8	2.04	94	1.50	86	1.48	79	1.15	69	0.67	112	0.15	91			1.52	48	0.25	90	1.00	90	0.85	74
23	SC	1.0	256	66	0.3	0.102	8.4	1.66	92	1.51	(75)	1.71	(84)	1.48	91	1.00	119	0.23	95	0.13	102	0.63	43	0.85	94	1.48	99		
24	SC	2.0	190	86	2.7	0.095	7.4	1.00	88	1.62	81	1.23	87	0.28	48	1.44	111	0.42	78	0.30	99	0.36	53					0.98	85
25	SC	6.0	134	75	4.0	0.094	7.7	1.30	91	1.04	78	0.90	80	0.73	63	1.32	116	0.81	97	0.50	106					1.06	87	0.00	84
26	SC	18.0	192	91	4.1	0.080		1.08	103	0.92	79		84																
Average				70			8.85		95		75.6		84.7		70.4		112.0		93.4		102.4		43.3		92.1		91		80.9

The amount of the injected K has been deducted from the calculated total K. Potassium contents (K) are expressed as milliequivalents per kilogram wet weight. Rats 4 to 7, 11 and 15 were anesthetized with urethane and no. 8 was treated with metrazol. No. 5 had one sciatic nerve cut. No. 6 had both denervated and one of them stimulated. No. 7 had one sciatic cut and stimulated; no. 4 had one intact sciatic stimulated. IP = intraperitoneal; SC = subcutaneous; ST = stomach tube. The majority of the muscle figures are averages of determinations on 2 different muscles. Figures in ( ) represent values assumed for purposes of calculation.

radioactivity and for potassium. The results of these eight experiments are summarized in table 3. Column 4 gives the total potassium per kilogram of whole rat. This figure was obtained from analyses of the carcass after adding in the weights and potassium contents of all the samples. The variation in different animals is surprisingly large and appears to be well beyond the experimental error. The average value is 66.4 m.eq. per kgm. or 0.26 per cent potassium. This is close to the value of 0.25 per cent reported by Heppel and Schmidt (1938), and also close to the average value of 69.8 m.eq. per kgm. for the total exchanged potassium as calculated in table 2. In most of the experiments the rat was kept in a metabolism cage so that the excreta could be collected and analyzed for radioactivity. The contents of the bladder at the time of

TABLE 3

Total balance and distribution of injected radioactive potassium

RAT NO.	TIME	ROUTE	TOTAL K	COUNTS RECOVERED	CARCASS COUNT	DISTRIBUTION IN PER CENT OF COUNTS INJECTED								
						Excreta	Liver	Skin	G.I.	Muscle	Blood	Heart	Brain	Kidney
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
	hours		*mM/kgm.*	*per cent*										
1	0.5	IP	62.0	78	69	10.4	11.4	8.7	16.5	28.4	0.6	0.79	0.05	0.76
2	0.5	IP	74.0	85	137	1.9	14.1	4.7	6.2	30.6	0.7	0.87	0.03	0.61
18	1.0	ST	60.9	61	57		14.7	6.0	13.2	22.4	0.8	0.62	0.05	1.36
13	3.0	IP		118	55	2.9	7.9	10.1		78.8	0.7	0.23		
25	6.0	SC	74.0	92	243	10.1	5.1	11.5	1.2	57.4	1.3	0.45	0.22	0.77
16	6.5	IP	56.6	89	512	9.0	6.7		4.6	84.8	1.1	0.45	0.09	0.28
17	10.0	IP	62.8	75	119	6.8	3.2	8.8	4.3	54.6	1.7	0.30	0.26	0.95
26	18.0	SC	74.3	85	481	9.2	3.0	8.9	4.7	52.6	2.7	0.31	0.31	0.64

IP = intraperitoneal; SC = subcutaneous; ST = stomach tube.

death as well as the contents of the gastro-intestinal tract were also included. This figure in per cent of the total counts injected per kilogram body weight is given in column 7 (excreta). There is no regularity in these figures because they include material not absorbed as well as material excreted both in feces and urine. In rat 18 the KCl was given by stomach tube and 44 per cent of the amount was found in the G-I tract at death. This figure was so large that (in this experiment) the distribution in the various tissues has been expressed in per cent of the amount absorbed.

The percentage of the total counts injected which were recovered either in the rat or in the excreta is given in column 5. On the average only 86 per cent was recovered. The large variations from this figure are probably due to errors in the counting because the variations are especially large

where the number of counts for a 5-minute period found in the carcass sample was especially low as shown in column 6. We are unable to account satisfactorily for the low average recovery of the injected counts. The amounts injected were measured with as much accuracy as a 5 ml. syringe permits.

In calculating the distribution of the injected counts in the various tissues listed in table 3, it was assumed that the skin represented 18 per cent and the muscles 40 per cent of the body weight. In the last experiment the skin was actually removed in toto and ashed and analyzed separately. In this case it amounted to 24 per cent of the body weight, but a similar procedure in experiment 2 gave a value of 17.9 per cent. In the other tissues actual weights of the whole liver, the whole G-I tract, etc., were obtained. On account of the assumption that 40 per cent of the body weight is muscle and the further assumption that all the muscles contained the same amount of K^{42} per gram as the gastrocnemius or rectus femoris muscles, which were the ones usually analyzed, the fraction of the injected dose allotted to muscle must be regarded only as a rough estimate. In some cases it is obviously too large for it amounts to nearly as much as all the counts recovered. For this reason the sum of columns 7 to 15 cannot be expected to add up to the value in column 5 for total counts recovered. It is evident, however, that muscles account for a larger fraction of the total counts than any other tissue.

In figure 1 the plasma potassium activities have been plotted against time. The scatter of the points is very large because each point represents a different rat. Intraperitoneal, subcutaneous and stomach tube experiments have all been included but no consistent differences are apparent. The plasma potassium activity rises immediately after the administration of the potassium but the active potassium is disposed of so quickly in the tissues by exchange or transport that the activity reached in the plasma is never much greater than the final equilibrium value. The graph shows also the scale for the amount of exchanged potassium which can be calculated by dividing the plasma value into 100. The graph indicates a maximum exchanged potassium of 95 m.eq. per liter whereas the total in the body is not over 70. This discrepancy is attributed to two different factors: 1, excretion of 5 to 10 per cent of the injected counts; 2, higher potassium activity in some of the tissues than in plasma due to incomplete mixing. Furthermore, if the activity were calculated in relation to the total counts found in the whole body instead of the total injected, the plasma K activity would have had nearly the expected value of about 1.4.

If the plasma activity be plotted instead of the plasma K activity the resulting curve is very similar in shape to that shown in figure 1. No significant differences between intraperitoneal and subcutaneous injections

are apparent and the final equilibrium value of the activity which is reached is about 0.1 with an average early peak value of 0.15. In one rat at 1 hour a maximum value of 0.2 was observed.

All these figures for activity and potassium activity of plasma refer, of course, to arterial blood and it is impossible to estimate how high the portal blood or the mixed venous blood may have been. The portal vein plasma must have been at least as high as the highest values for the K activity observed in the liver.

The similarity between the curves for plasma activity and plasma potassium activity when plotted against time indicates that there was little change in the concentration of potassium in the plasma even immedi-
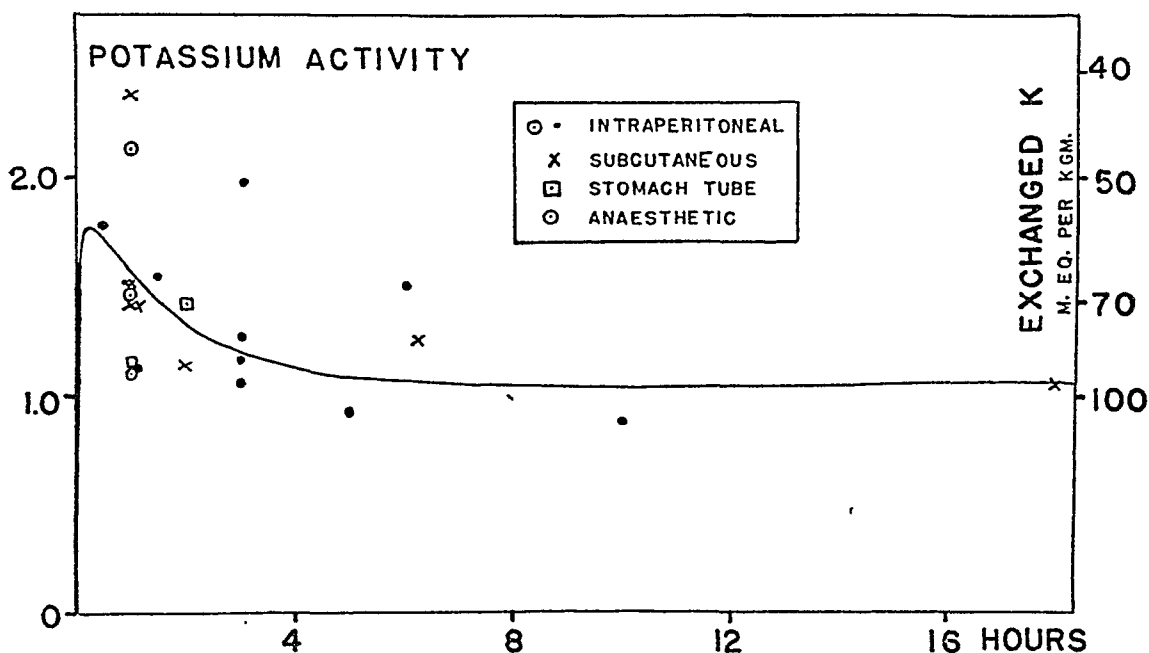
Fig. 1. Potassium activity of plasma and calculated exchanged potassium for various methods of injection.

ately after injection. Our data do not include figures for plasma K previous to injection but the rats killed within 1 hour after injection,gave values only about 2 m.eq. per kgm. larger than the rats killed many hours later.

The figures presented for a few experiments in table 3 show that the injected potassium is found in continuously increasing amounts in muscles, brain and blood cells while the amount in the liver at least appears to pass through an early maximum and then to decrease. In general, a mobile fraction amounting to about 15 per cent of the injected radioactive K is lodged first in the viscera and then gradually moves over into the muscles and skin as mixing becomes complete. This same result appears from the

curves of figure 2 in which the relative potassium activity of liver, muscle, brain, and erythrocytes is plotted against time after injection. (This is the same as the potassium activity of the tissues corrected to a plasma potassium activity of 1.0.) The muscle curve shows a gradual rise until the relative potassium activity is equal to or slightly greater than the plasma value, whereas the liver curve rises quickly to values much greater than 1 and then slowly falls. When all curves have reached the plasma value the relative potassium activity is the same throughout the body and
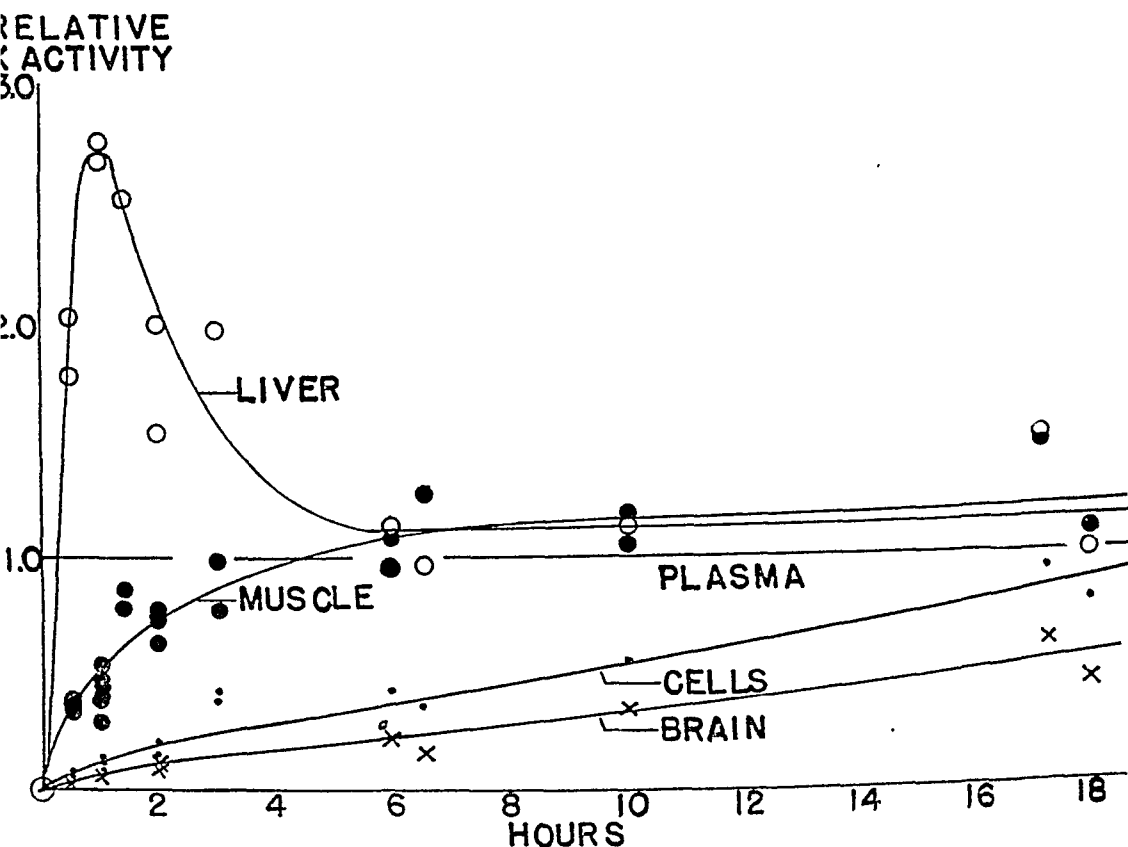
Fig. 2. Relative potassium activity of liver (only intraperitoneal administration plotted during first 4 hours), muscle, erythrocytes (cells), and brain. Value of 1 indicates complete equilibrium.

mixing may be said to be complete. We consider it likely that the muscle curve does go slightly above unity and then returns slowly to that value. In any event mixing is nearly complete in 4 to 6 hours so far as muscle and liver are concerned. Brain and erythrocytes rise even more slowly than muscle. The meaning of the liver curve will be discussed more completely later. It is sufficient to emphasize here that 1 hour after injection the liver may contain twice as many counts per mol of potassium as does the plasma. This indicates either a high degree of permeability to potassium

or a large mass movement of highly radioactive potassium from the plasma to the liver or both.

The liver and the muscle represent two categories into one of which all the tissues of the body appear to fall. In figure 3, for example, are plotted similar curves for the heart and the skin, the former behaving like the liver
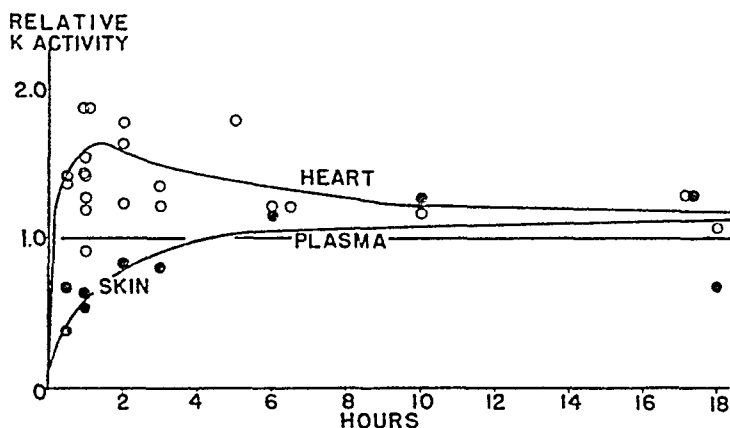
Fig. 3. Relative potassium activity of heart and skin. Value of 1 indicates complete equilibrium between tissue and plasma.
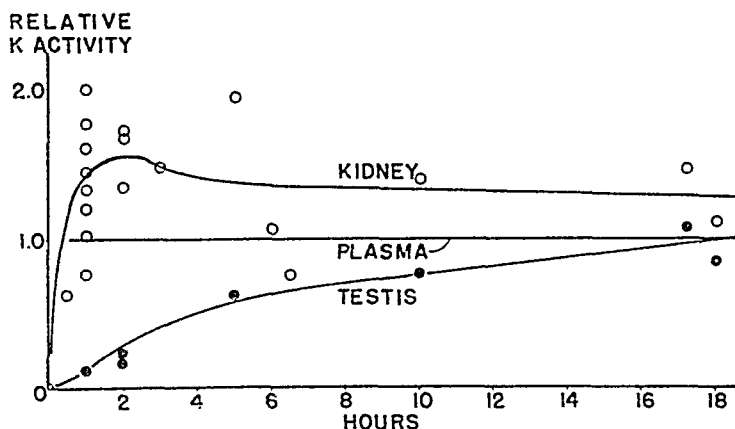
Fig. 4. Relative potassium activity of kidney and testis. Value of 1 indicates complete equilibrium between tissue and plasma.

and the latter like the muscle. Likewise in figure 4 the kidney behaves like liver while testis resembles muscle.

It was thought possible that the high activities observed in the liver after intraperitoneal injection might be due to contamination from unabsorbed potassium in the peritoneum during dissection. In some experiments, therefore, we washed out the peritoneum and analyzed the washings separately without finding more than a few per cent of the injected dose present after 30 or more minutes and only 15 per cent after

10 minutes. After intraperitoneal injection, the absorbed material would pass first through the liver from the portal circulation so that this tissue would be exposed to a plasma with a higher potassium activity than any other tissue in the body. To avoid this situation a number of animals were injected subcutaneously and, in two cases, by stomach tube. The results of subcutaneous injection differ from those with intraperitoneal injection only in the case of liver, gastro-intestinal tract, diaphragm, and possibly lung. Administration by stomach tube yields results not differing significantly from those found after intraperitoneal injection. Figure 5 shows these differences. In all cases, intraperitoneal or stomach tube administration leads to higher relative potassium activities, especially for the short time intervals. The reasons for this are that the liver, alimentary canal, and diaphragm enjoy favorable anatomical positions with regard to the venous and lymphatic drainage from the peritoneal cavity and therefore would be in contact with a solution richer in K^{42} than would tissues less favorably situated. In the case of the lung it seems probable that the difference is not significant.

DISCUSSION. Radioactive potassium in the plasma will disappear into the tissues: a, by a possible mass movement or transport of potassium in solution, that is, an ingestion of an intracellular potassium solution by cells, or b, by exchange of radioactive potassium in the plasma with normal potassium in the cells. There will also be an excretion of labelled potassium in the urine, but this will be relatively small because the radioactive potassium is so quickly mixed with most of the body potassium and the absolute amount of potassium excreted while this is happening is so small compared to the total in the body.

The participation of both these processes of transport and exchange is clearly shown by the data. Thus the potassium level in the plasma is not much increased in spite of the absorption of an amount of potassium equal on the average to 15 times the total plasma potassium. Since the absorption is nearly complete in 15 minutes it is clear that the transport of an equal amount of potassium into the tissues is also complete in this time. According to previous data, injected potassium appears to distribute itself in the body in proportion to the body water. (Winkler and Smith, 1938; Wilde, 1938; Fenn, 1939.) The expected increase in plasma potassium is therefore only about 2 mM per liter which is not far from the observed increase. Without any transport into cells and with all the injected potassium confined to the extracellular spaces the increased concentration in the plasma would have been 2 to 3 times as large.

Clear evidence that K^{42} must also disappear from the plasma by exchange for K^{39} from the tissues is found in the fact that the potassium activity rises quickly to a maximum *and then decreases*. A transport of mixed plasma potassium into the tissues (without exchange) could decrease the

activity of the plasma but could not alter the potassium activity which would increase to a maximum during absorption of K^{42} and would not decrease unless potassium of lower potassium activity entered either from ingested food or by exchange from the tissues, or unless the tissues took up K^{42} in preference to K^{39}.

While both transport and exchange contribute to the distribution of radioactive K in the body it is noteworthy that by the process of transport
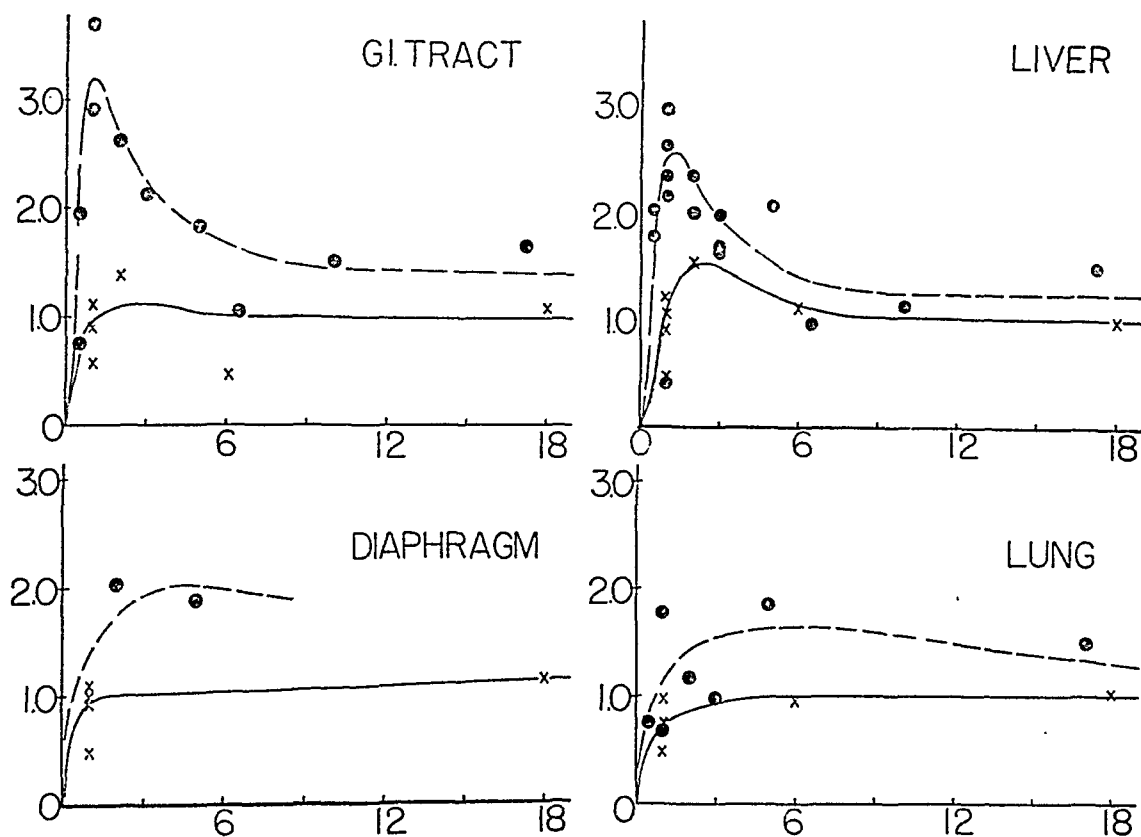
Fig. 5. Ordinates—relative potassium activity of liver, gastro-intestinal tract, diaphragm, and lung after intraperitoneal and stomach tube (● —— ● ——) and after subcutaneous (× —— × ——) administration. Value of 1 indicates complete equilibrium between tissue and plasma. Abscissae—time in hours.

the K^{42} would probably be distributed evenly in all the body water whereas by the process of exchange it would be distributed evenly in all the body potassium. Since the latter is the observed result it is evident that complete exchange does eventually occur even though the excess load of K injected is distributed according to a different rule.

We may now consider what changes in tissue activity are to be expected simply as a result of ionic exchange of K^{42} for K^{39}. Since the potassium activity of the plasma passes through a maximum and then falls it is to

be expected that the tissues will tend to do likewise. Those tissues which are most permeable to potassium will also show a considerable peak in potassium activity, but this peak will lag somewhat behind the plasma peak whereas tissues which are relatively impermeable to potassium will show scarcely any peak, and this will occur very late. Since the rate of change of the activity in any tissue will be zero when the potassium activity inside is just equal to that outside it is evident that the peaks in potassium activity shown by the various tissues should fall exactly on the curve for potassium activity in the plasma.[1] Unfortunately our present data are scarcely adequate to enable us to decide whether they conform to this theoretical condition or not, because there are too few experiments at very short durations, and the scatter of the points is too great. Moreover we have no data for the portal blood plasma which is necessary for comparison with liver.

On the other hand a transport of radioactive K into the tissues could also cause tissue potassium activities greater than simultaneous plasma values if the transport were greater into some tissues, such as liver, than into others such as muscles. The difference would be enhanced if the portal blood plasma entering the liver had a higher K activity than arterial plasma which feeds the muscles.

Evidence for greater transport of K into liver than into muscles was obtained in this laboratory (Fenn, 1939) by analyzing cat livers before and after injection of KCl. It was concluded, subject to certain limitations, that the liver, which accounts for only about 3 per cent of the body weight, was nevertheless able to absorb 7 to 15 per cent of the injected K. Two similar experiments have been performed on rats and the data are shown in table 4. The rats were etherized and samples of the liver were removed for analysis. Immediately after recovery from the ether 5 ml. of $\frac{m}{10}$ KCl was given to each rat by stomach tube. Two hours later both rats were killed, and samples of blood and liver were taken for analysis. The liver was thus analyzed before and after KCl for K, H_2O and blood content and the blood was analyzed for K. In interpreting the results it was assumed that the total weight of dry liver did not change. The wet weight of the liver before injection was calculated from that found after injection on this basis. The amount of blood in the liver was determined by mincing a small piece of liver in a measured volume of isotonic salt solution and counting the red cells. Red cell counts were also made in whole blood. These data permitted a correction of the total liver K for blood K. The results of the 2 experiments show that 7.6 and 6.4 per cent respectively of the potassium dose was located in the liver. Chloride

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[1] A more mathematical application of this theory to red blood corpuscles will be found in a paper by Dean, Noonan, Haege and Fenn (1940).

spaces were determined also in experiment 1 and found to be somewhat less after injection. A correction for extracellular K, therefore, would increase still further the calculated percentage of the dose found in the liver. These experiments, therefore, support the idea that the high potassium activities in the liver are due in large measure to transport or mass movement of K from a portal blood plasma of high potassium activity. The process of exchange may well be a much slower process, thus accounting for the ease with which the K activity of the plasma can fall below that in the liver, heart, and other viscera. It is even possible that exchange is no more rapid in liver than in muscle, all the difference between the tissues being accounted for by transport of material of high K activity from the

TABLE 4

Potassium in rat liver before and 2 hours after giving 5 cc. of m/10 KCl by stomach tube

	EXPT. I		EXPT. II	
	Before	After	Before	After
Weight of liver, wet*..........	8.17	8.38	10.05	9.96
K, m.eq. per kgm. wet........	99.3	100.2	99.3	102.5
K, m.eq. per kgm. dry........	320	331	324	331
Dry weight, per cent..........	31.03	30.26	30.61	30.97
Blood, per cent..............	5.15	2.99	3.7	1.65
K in blood, m.eq. per kgm.....		53.0		57.8
Total K minus blood K, m.eq..	0.789	0.827	0.979	1.011
Excess K after injection, m.eq..		0.038		0.032
Excess in per cent of amount fed		7.6		6.4

* The weight before injection was calculated from the dry weight of the liver found after KCl. The body weights were 245 and 248 grams respectively in experiments 1 and 2. In experiment 1, the chloride space of the liver was 28.8 per cent before and 22.2 per cent after injection, the K contained in this space being 0.018 and 0.014 m.eq. respectively. The blood in the liver was lower after injection because the samples were taken after bleeding the animal to death.

portal blood plasma. In a preliminary abstract of this work (Noonan et al., 1940) we interpreted our results in this sense but further consideration shows that this view is not yet completely proved. The relative quantitative importance of exchange and transport in the results must still be measured.

<center>SUMMARY</center>

At different times after administration in various ways of radioactive potassium to albino rats the animals were killed and samples of plasma, red cells, and various tissues were analyzed both for total potassium and for radioactivity. The radioactive potassium was found to penetrate rapidly into most of the tissues of the body, relatively little being found

in the plasma.   The rate of penetration is highest in liver, heart, kidney, lung, diaphragm, and gastro-intestinal tract, intermediate in muscle and skin, and low but not absent in testes, erythrocytes and brain.

In tissues where penetration is rapid the activity per mol. of total potassium present in the first 1 to 2 hours is 1.5 to 2.5 times as high as the simultaneous plasma value.   The difference was especially great after intraperitoneal injection, but was still present after subcutaneous injection.

Since the increase in total K in the plasma is only a small fraction of the total K injected, elimination of K from the blood must have occurred by mass movement of K or by exchange with some other cation.   The low value of the activity in the plasma on the other hand indicates a nearly complete mixing of radioactive K with all the normal K in the body. Both processes indicate widespread permeability to K.

After equilibrium is established the bulk of the radioactive potassium is found where the bulk of the total body potassium is, i.e., in the muscles, with skin and viscera next in importance.

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MEASUREMENT OF THE BLOOD FLOW OF THE LIVER

JOHN H. GRINDLAY, J. F. HERRICK, AND FRANK C. MANN

From the Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota

The rate of flow of blood through the liver was measured in dogs by the thermostromuhr method. The measurements of blood flow included the flow in the hepatic artery, portal vein and thoracic inferior vena cava, the abdominal inferior vena cava having previously been ligated. The study was made in order to obtain data on the inflow of blood to the liver and outflow of blood from it as a basis for further studies on trained dogs under various physiologic conditions.

The flow of blood from the hepatic veins has been determined in several investigations by means of a mechanical stromuhr. The average value for anesthetized dogs has been reported to be 84 cc. per minute per 100 grams of liver by Burton-Opitz (1), and 64 to 144 cc. per minute per 100 grams of liver by Macleod and Pearce (2). In anesthetized cats Barcroft and Shore (3) found the flow to be 33.3 to 48 cc. per minute and Schmid (4) reported a value of 54 cc. per minute per 100 grams of liver. Blalock and Mason (5) collected and measured the blood flowing through the hepatic veins in dogs weighing from 11.5 to 18.8 kgm. under local anesthesia; they reported the flow to be 216 to 612 cc. per minute, averaging 387 cc. per minute (27 cc. per minute per kilogram of body weight or 82 cc. per minute per 100 grams of liver). Grab, Janssen and Rein (6, 7) measured the total hepatic blood flow in dogs after recovery from anesthesia with the thermostromuhr. The average flow was 65.3 cc. per minute per 100 grams of liver. The average flow through the thoracic inferior vena cava in five dogs ranged from 250 to 860 cc. per minute, while the flow through the abdominal inferior vena cava ranged from 60 to 340 cc. per minute. Soskin, Essex and two of us (Herrick and Mann) (8) used the thermostromuhr and reported the flow through the thoracic inferior vena cava of anesthetized dogs, when the abdominal inferior vena cava was ligated, to be from 40 to 160 cc. per minute per 100 grams of liver. Using the same method and preparation, Essex, Baldes and two of us (Herrick and Mann) (9) found the flow through the thoracic inferior vena cava in a trained dog weighing 7.8 kgm. to be 408 to 552 cc. per minute.

Average values for flow of blood in the hepatic artery of anesthetized dogs have been 143.4 cc. per minute (10), 15 to 55 cc. per minute (11),

and 30 to 65 cc. per minute in dogs after recovery from anesthesia (6). Similar values for blood flow in the portal vein of anesthetized dogs have been 268.2 cc. per minute (1), 80 to 200 cc. per minute (11), 100 to 330 cc. per minute (12), and 125 to 340 cc. per minute in dogs after recovery from anesthesia (6).

METHOD.   Ligation of the inferior vena cava between the entrance of the hepatic veins and that of the right lumbo-adrenal vein had been performed on all the dogs several months previously.   Usually this operation was done in two stages but in an occasional experiment evidence of sufficiently developed collateral veins permitted completion of the ligation to be delayed until the time of the experiment.   The weight of the dogs varied from 11.5 to 25.5 kgm. and averaged 16.76 kgm.   The flow of blood in the hepatic artery, portal vein and thoracic inferior vena cava was measured by the thermostromuhr method (13).   Thermostromuhr units were placed on these·vessels while the dogs were under anesthesia.   The units, which ranged from 2 to 6 mm. in diameter, were calibrated in vitro on several occasions before and after an experiment and were found to be reliable within the limits of accuracy of the method.   When blood flow was studied while the dogs were under anesthesia, pentobarbital sodium was used as the anesthetic agent.   Ether anesthesia and aseptic technic were used for applying the units in those animals in which the blood flow was studied after recovery from anesthesia.

A midline or right rectus incision afforded access to the portal vein and hepatic artery.   Care was taken to avoid enlarged collateral veins in the abdominal wall.   The pancreaticoduodenal artery and vein were ligated in the critical experiments, the former at its origin from the hepatic artery and the latter at its junction with the portal vein.   A thermostromuhr unit was placed on the hepatic artery proximal to the ligated pancreaticoduodenal artery in the critical experiments.   This procedure was the most difficult and time consuming, great care being required in the freeing of the hepatic artery from its investment of splanchnic nerves.   A few nerve fibers were unavoidably traumatized.   The portal vein unit was applied caudal to the point of entry of the ligated pancreaticoduodenal vein when liver blood flow was measured during anesthesia.   When flow was measured after recovery from anesthesia, the unit was placed on the portal vein above the pancreaticoduodenal vein.

The abdominal incision was then closed and artificial respiration under positive pressure was started.   The right pleural cavity was opened by an incision in the sixth thoracic interspace, and the thoracic inferior vena cava exposed.   Freeing of the middle portion of this vessel and application of a thermostromuhr unit were accomplished very quickly.   The chest wound was then closed and artificial respiration discontinued except in a few of the experiments in which blood flow was measured during anesthesia.

The flow of blood was recorded on a moving photographic film. By this means it was possible to determine accurately the flow of blood in several vessels simultaneously and continuously. However, a few direct

TABLE 1

Hepatic blood flow of a group of dogs during pentobarbital sodium anesthesia*

DOG	WEIGHT	NUMBER OF OBSERVA-TIONS	BLOOD FLOW IN CUBIC CENTIMETERS PER MINUTE			
			Hepatic artery	Portal vein	Inflow (hepatic artery plus portal vein)	Outflow (thoracic inferior vena cava)
	kgm.					
1	11.8	11	95	321	416	Not measured
2	15	1	(122)	(230)	(352)	(435)
		1	(88)	(200)	(288)	(292)
		5	55.4	248	303.4	252.4
		1	(75)	(145)	(220)	(267)
		5	86	151.8	237.8	269
3	18.8	1	(105)	(450)	(555)	(500)
		15	106	393	499	447
		1	(110)	(505)	(615)	(465)
		1	(99)	(420)	(519)	(565)
		8	102	345	447	455
		1	(105)	(365)	(470)	(520)
4	16.5	1	(163)	(218)	(381)	(390)
5	18.0	1	(112)	(365)	(477)	(460)
		7	51	335	386	351
		1	(53)	(435)	(488)	(465)
		9	44.6	407.4	452	478.7
		1	(47)	(420)	(467)	(370)
6	11.5	1	(61)	(203)	(264)	(344)
7	25.5	1	331	260	591	620
8	17.0	1	(440)	(130)	(570)	(590)
		11	478.5	100.2	578.7	557.4

* When a series of simultaneous observations were made, the table gives the number of such observations and the average value of the blood flow in each vessel during the short interval covered by the observations. The series of eleven observations on dog 8 were made at half minute intervals; at other times a series of observations represented simultaneous readings at one minute intervals. Single observations are shown enclosed in parentheses in the table in order to indicate that the observations were from three to five minutes apart. The observations on dogs 2 and 5 were made with the chest open and with positive pressure artificial respiration.

observations of flow in each vessel were always made and such observations were not made simultaneously.

RESULTS. During pentobarbital sodium anesthesia the usual blood flow ranged from 44.6 to 163 cc. per minute in the hepatic artery, and from 145 to 505 cc. per minute in the portal vein (table 1). Although the portal

vein flow was usually two to three times that of the hepatic artery, two dogs (dogs 7 and 8, table 1) had larger hepatic artery flows than portal vein flows.   The blood flow in the thoracic inferior vena cava of the group of anesthetized dogs ranged from 252.4 to 620 cc. per minute (table 1). After recovery from ether anesthesia, the usual flow in the portal vein of a group of dogs ranged from 206.5 to 473.9 cc. per minute, and from 368 to 507.5 cc. per minute in the thoracic inferior vena cava (table 2).   The portal vein flow and thoracic inferior vena cava flow were measured simultaneously in some experiments after recovery from anesthesia (dogs 9 and 11, table 2), and the hepatic artery flow was calculated by subtraction of portal flow from thoracic inferior vena cava flow.   The values of hepatic artery blood flow thus obtained were 33.6 and 65 cc. per minute.

TABLE 2

Blood flow in the portal vein and thoracic inferior vena cava in a group of dogs on the first or second day after recovery from ether anesthesia*

DOG	WEIGHT	DAY	FLOW IN CUBIC CENTIMETERS PER MINUTE		CALCULATED HEPATIC ARTERY FLOW
			Portal vein	Thoracic inferior vena cava	
	kgm.				
9	17.5	1	303	368	65.0
10	19.4	1	206.5		
		2	330		
11	22.0	2	473.9	507.5	33.6
12	21.4	1		505	

* The data represent typical values obtained on the first and second days after operation. Both the portal vein and thoracic inferior vena cava blood flow were measured in dogs 9 and 11.   The calculated hepatic artery flow for these dogs represents the difference between the thoracic inferior vena cava flow and portal vein flow.

When the rate of flow of blood was measured in two or more vessels simultaneously by a photographic recording of the flow in these vessels, it was observed that the flow in each vessel was constant within certain limits.   This has been illustrated by a typical experiment on one dog (dog 3, table 3) in which simultaneous measurements of hepatic artery, portal vein and thoracic inferior vena cava flow at one minute intervals show no wide fluctuations.   However, subtraction of outflow from total inflow revealed that there were small differences at each interval, and that frequently the magnitude of these differences increased and decreased in cyclic fashion.   When simultaneous measurements of inflow and outflow over a period of time were plotted graphically, cyclic changes in inflow and outflow, which were out of phase with each other, could be seen

(fig. 1). This suggests that the differences between inflow and outflow were caused by rhythmic storage and discharge of stored blood by the

TABLE 3

Dog 8: Pentobarbital sodium anesthesia. Blood flows in the hepatic artery, portal vein and thoracic inferior vena cava were recorded simultaneously at one minute intervals for a period of eight minutes

TIME	FLOW IN CUBIC CENTIMETERS PER MINUTE			
	Hepatic artery	Portal vein	Inflow (hepatic artery plus portal vein)	Outflow (thoracic inferior vena cava)
minutes				
1	102	340	442	440
2	99	355	454	440
3	102	355	457	420
4	99	345	444	465
5	105	355	460	440
6	95	340	435	450
7	107.5	330	437.5	465
8	107.5	340	447.5	520
Average.......	102	345	447	455
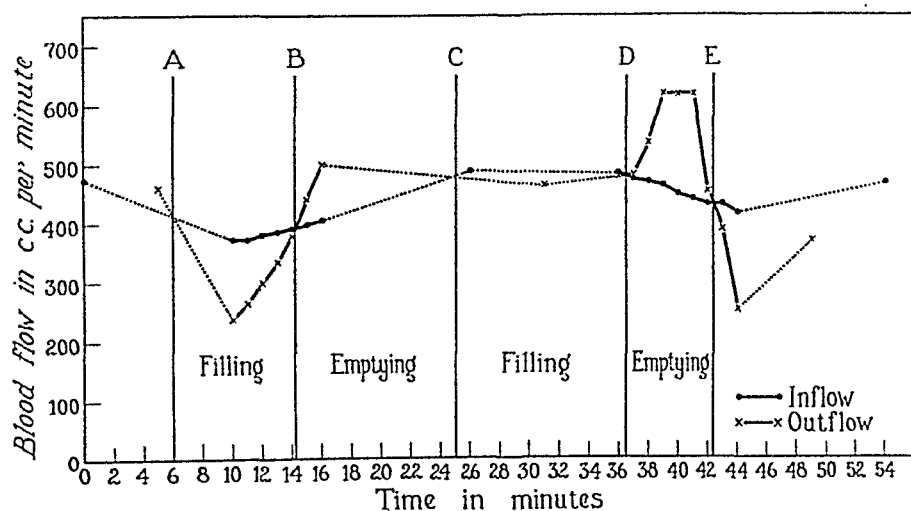
Fig. 1. Dog 5: pentobarbital sodium anesthesia. Liver inflow and outflow during an interval of about fifty minutes. Inflow of blood was computed by addition of values for simultaneous observations of flow in the hepatic artery and portal vein. The outflow of blood was measured in the thoracic inferior vena cava. Inflow exceeded outflow during the A-B and C-D intervals, and outflow exceeded inflow during the B-C and D-E intervals. Broken lines indicate intervals during which blood flow was not recorded photographically.

liver. In the presence of this phenomenon it is evident that inflow and outflow would only occasionally be equal in magnitude.

COMMENT. After each experiment was concluded examination of the abdominal inferior vena cava at the site of ligation revealed that the occlusion had been complete. There was no evidence that total inflow through the portal vein or the hepatic artery was not measured. Small veins in the central portion of the diaphragm entering the thoracic inferior vena cava above the liver were noted but they could not have contributed greatly to measurements of liver outflow. Occasionally these small veins were ligated.

Measurement of the blood flow of the liver by the technic used in this study has given values which are of approximately the same magnitude as values of previous studies on dogs. The flow of the hepatic artery during pentobarbital sodium anesthesia in six experiments ranged from 44.6 to 163 cc. per minute. The calculated flow of the hepatic artery after recovery from ether anesthesia ranged from 33.6 to 65 cc. per minute. These values may be compared with values of 143.4 cc. per minute (Burton-Opitz, 10), 30 to 65 cc. per minute (Grab and co-workers, 6, 7), and 15 to 55 cc. per minute (Schwiegk, 11). The flow of the portal vein during pentobarbital sodium anesthesia in six experiments ranged from 145 to 505 cc. per minute. The usual portal vein flow after recovery from anesthesia ranged from 206.5 to 473.9 cc. per minute. Others have obtained values of 268.2 cc. per minute (Burton-Opitz, 1), 125 to 340 cc. per minute (Grab and co-workers, 6, 7), 80 to 200 cc. per minute (Schwiegk), and 100 to 330 cc. per minute (Katz and Rodbard, 12).

In two experiments (dogs 7 and 8, table 1) it will be noted that the relationships of the blood flow in the hepatic artery and the portal vein are reversed in respect to the values for flow in these vessels of other dogs of the study. Although the data do not explain the cause of the reversed relationship, they support the hypothesis that the hepatic artery under certain conditions may compensate for a lowered supply of portal vein blood to the liver. Burton-Opitz (1, 10) estimated the hepatic artery to carry about 30 per cent and the portal vein about 70 per cent of the liver blood flow. He (14, 15, 16) expressed the opinion, however, that there was a free reciprocal interchange between these two flows and that the hepatic artery flow compensated for low portal flow, the liver radicles of both vessels possessing motor activity. Macleod and Pearce observed that occlusion of the hepatic artery decreased liver blood flow about 30 per cent, of the portal vein about 60 per cent. However, they expressed the opinion that this relationship was variable and controlled by vasomotor activity, feeble in the case of the radicles of the portal vein in the liver. Grab, Janssen and Rein estimated the flow in the hepatic artery to be from 12 to 22 per cent of the total blood flow of the liver, while Schwiegk estimated it to be from 20 to 25 per cent, and Blalock and Mason estimated it to be from 12.6 to 24.5 per cent. Schwiegk found that warming of the

dog caused an appreciable increase of hepatic artery flow and decrease of portal vein flow. Soskin and co-workers (8) have stated that the share of blood entering the liver carried by the portal vein or hepatic artery may vary as much as from 10 to 90 per cent in either vessel.

Calculation of inflow of blood to the liver, by addition of hepatic artery and portal vein blood flow values, should give a result which closely approximates the measured outflow of blood through the thoracic inferior vena cava. In general, the outflow and inflow values were found to be in close agreement. However, differences in such values occurred and these were analyzed. If differences between inflow and outflow were due entirely to inaccuracy of the method of measurement of flow, one would expect random variations in the magnitude of the differences in a series of inflow and outflow observations. On the other hand, a consistent trend in magnitude might indicate a storage of blood or discharge of stored blood by the liver. An actual comparison of consecutive pairs of inflow and outflow observations disclosed the fact that differences usually had a certain relationship. This relationship indicated alternate storage and discharge of blood by the liver. A typical experiment illustrating such data has been presented in table 3 where an initial phase of filling of the liver appears to be followed by a phase of emptying. Similar data covering a longer interval have been presented graphically in figure 1. During such conditions it is obvious that the outflow of blood through the thoracic inferior vena cava would only at certain times be of the same magnitude as the combined portal vein and hepatic artery flow.

From this study it appears to us that the rate of blood flow of the liver is subject to many physiologic variations, such as rhythmic filling of the hepatic sinusoids. For this reason a correlation of liver blood flow with the size of the liver or animal is not always apparent and, indeed, should not be expected. Continuous observation over a period, as the thermostromuhr method permits, discloses wide physiologic variations. We have made similar observations using this method in studies of blood flow in other viscera. A single observation represents only the rate of blood flow under the conditions of the moment at which the observation was made. Because of its dynamic nature we believe that it is fallacious to represent liver blood flow as a static flow, in terms of the average flow for an animal, or in terms of rate of flow for a unit of liver or body weight.

The data obtained in this study merit one further consideration. While measurements of blood flow in the hepatic artery, portal vein and thoracic inferior vena cava by the thermostromuhr method show variations in the flow of these vessels, such variations do not incriminate the method. In general, simultaneous observation of flow in all these vessels discloses that wide fluctuations of flow in one vessel are reflected in compensatory changes in the flow of the other vessels. Smaller fluctuations in flow appear to be

concerned with rhythmic filling of the liver with blood. It is our belief that these consistently observed phenomena lend support to the reliability of this method for study of hepatic blood flow.

SUMMARY

The rate of flow of blood in the hepatic artery, portal vein and thoracic inferior vena cava was measured in a group of dogs by the thermostromuhr method. The abdominal inferior vena cava had previously been ligated. During pentobarbital sodium anesthesia the usual flow ranged from 44.6 to 163 cc. per minute in the hepatic artery, from 145 to 505 cc. per minute in the portal vein, and from 252.4 to 620 cc. per minute in the thoracic inferior vena cava. When total inflow and outflow of blood from the liver were measured, the values obtained were in close agreement.

After recovery from ether anesthesia the usual flow in the portal vein of a group of dogs ranged from 206.5 to 473.9 cc. per minute, and from 368 to 507.5 cc. per minute in the thoracic inferior vena cava. The calculated hepatic artery flow ranged from 33.6 to 65 cc. per minute.

Low blood flow in the portal vein in two anesthetized dogs was accompanied by high blood flow in the hepatic artery.

Simultaneous observations of inflow and outflow indicate that under certain conditions the liver may alternately store blood and discharge stored blood.

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THE FORMATION OF HYDROXYTYRAMINE BY EXTRACTS OF RENAL CORTEX AND BY PERFUSED KIDNEYS

R. J. BING

With the assistance of E. COHEN

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

In 1939, Holtz and co-workers (1) showed that the anaerobic incubation of renal cortical extracts with 1-dopa (1-dihydroxyphenylalanine)[1] resulted in the formation of a pressor substance which was identified as hydroxytyramine. According to these investigators the formation of this substance from dopa was caused by a decarboxylase and took place only under oxygen lack. Under aerobic conditions, however, hydroxytyramine was quickly oxidized by an amino-oxidase to dioxyphenylacetaldehyde, a depressor substance. The fact that an oxygen-free atmosphere was necessary for the formation of hydroxytyramine suggested the possibility that a similar reaction takes place in kidneys in situ, made ischemic by Goldblatt's method (2). Therefore, experiments were performed to investigate the production of hydroxytyramine from dopa in isolated cat's kidneys perfused with a reduced blood flow. These results were compared with those obtained by the incubation of dopa and extracts of renal cortex under anaerobic conditions.

METHODS. The method of Holtz (1) was employed in the preparation of hydroxytyramine by incubation of dopa and renal extracts. Guinea-pig kidneys were used, as they are rich in dopa-decarboxylase and thus are more active than those of other species in forming the pressor substance. Twelve grams of cortex from three animals were ground in a mortar with quartz sand and extracted for five minutes with 125 cc. of M/20 disodium phosphate buffer (pH 7.4). The mixture was then centrifuged for fifteen minutes and the supernatant fluid mixed in an Ehrlenmeyer flask with 125 mgm. of dopa dissolved in 10 cc. of distilled water. After adding a few cubic centimeters of toluene, nitrogen was bubbled through the solution for five minutes. Then the flask was sealed and the solution was incubated at 39°C. for eight hours. At the end of this time, 7.5 cc. of 50 per cent trichloracetic acid were added and the solution was filtered with suction.

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[1] Hoffman-La Roche.

The protein-free filtrate was extracted three times with ether to remove excess trichloracetic acid. Finally, the solution was distilled to dryness in vacuo at 60°C., the residue dissolved in 10 cc. of water, neutralized with NaOH, and used for injection.

Cats anesthetized with nembutal (0.6 cc. per kgm. body weight, intraperitoneally) were used in all experiments. In the perfusion experiments, an apparatus was used which permitted parallel perfusion of several organs under pulsatile pressure in a recirculating system. The perfusate consisted of heparinized blood. The apparatus will be described in detail in a further communication.

EXPERIMENTS. *Experiments with kidney extracts.* In six experiments, 1 cc. of the protein-free solution made from 12 grams of renal cortex was injected into a test cat. In one case the blood pressure rose from 140 to 230 mm. Hg, the rise lasting for three and one-half minutes (fig. 1). Similar results were obtained in the other five experiments. The injection of 6 mgm. of dopa dissolved in 1 cc. of Ringer's solution had no effect. It will be seen from figure 2 that, after injection of cocaine hydrochloride (6 mgm. per kgm. body weight, intravenously), the pressor effect of the kidney extract was enhanced. Since Holtz (1) has shown that cocaine potentiates the action of hydroxytyramine, it is possible that the rise in blood pressure resulting from the injection of the kidney extract was caused by hydroxytyramine.

In three experiments the influence of oxygen upon the production of hydroxytyramine from dopa by renal cortex was investigated. The disodium phosphate buffer extract of 16.2 grams of guinea pig cortex was divided into three samples of equal volume. The first sample was incubated under nitrogen with 65 mgm. of dopa dissolved in 20 cc. of water; the second, with the same amount of dopa under oxygen; and the third, without dopa under nitrogen. The samples were left in the incubator for eight hours at 38°C. After deproteinizing, distilling to dryness, redissolving and neutralizing the solutions, 1 cc. of each portion was injected into the same animal and the blood pressure recorded. The injection of the extract incubated under nitrogen caused a rise in blood pressure of 140 mm. Hg, lasting for approximately three minutes; that of the extract incubated under O_2, of 75 mm. Hg. One cubic centimeter of the third portion produced only a slight rise. A second injection of the same sample, however, caused a fall in blood pressure.

From the results obtained with the solution incubated under oxygen it was apparent that under aerobic conditions the production of hydroxytyramine was diminished but not abolished. Since it has been claimed by Holtz and co-workers (1) that in the presence of renal extract and oxygen an enzymatic breakdown of hydroxytyramine occurs, it was considered possible that the discrepant result mentioned above was caused by an

overproduction of hydroxytyramine due to the presence of an excess of dopa.   In this case not all of the amine could be oxidized to the aldehyde.

To investigate this problem 82 cc. of phosphate buffer extract made from 13.6 grams of renal cortex were divided into two equal parts.   To each sample 35 mgm. of dopa were added.   The first solution was kept under nitrogen; the other under oxygen.   After incubation for eight hours the proteins were removed, the extracts distilled to dryness, and the residues were finally dissolved in 6 cc. of water in each case and then neutralized. In three experiments the injection of 2 cc. of the portion derived from the aerobic kidney extract caused no change in blood pressure, whereas an equal amount of the anaerobic extract had a pressor action.   These experiments confirmed the observation of Holtz that a quick breakdown of hydroxytyramine takes place in the presence of renal cortex and oxygen, provided that the amount of dopa does not exceed the capacity of the amino-oxidase in the renal tissue.

Experiments on perfused organs.   In order to ascertain whether the whole kidney has the capacity, possessed by renal cortical extracts, to transform dopa into hydroxytyramine, eight experiments were performed on the isolated organ.   The kidney was excised according to the method of Steggerda, Essex and Mann (3).   The perfusate consisted of 100 cc. of heparinized blood.   The ventilated lung was included in the perfusion circuit to remove the constrictor substances normally present in shed blood (4).   After constant blood flow through the kidney had been established, the rate of flow through that organ was reduced by partial occlusion of the inflow tube leading to the renal artery.   This was accomplished by manipulation of the stopcock in that part of the perfusion apparatus. The pressure head in front of the occlusion was kept at 120/100 mm. Hg.

After forty-five minutes of perfusion 15 cc. of the perfusate were removed to serve as a control, and 200 mgm. of dopa, dissolved in 30 cc. of Ringer's solution, were added to the perfusion fluid.   Perfusion then continued for an additional period of two hours.   After that time the perfusate, as well as the control previously removed, were centrifuged for fifteen minutes and the plasma injected into a cat.

In one experiment the blood flow through the kidney was reduced from approximately 15 to 5 cc. per minute.   Five cubic centimeters of plasma, obtained by centrifuging the kidney perfusate, were injected into the animal.   Following the injection, the blood pressure rose from 100 to 220 mm. Hg, the rise lasting for four minutes (fig. 3).   After the injection of cocaine hydrochloride (6 mgm. per kgm. body weight, intravenously), the injection of 5 cc. of the same plasma caused a rise of 125 mm. Hg, lasting for eight and one-half minutes.   The control plasma had no effect.

In a second experiment, the rate of flow through the kidney was reduced from 15 to 8 cc. of blood per minute.   The injection of 5 cc. of perfused

plasma raised the blood pressure from 100 to 190; after injection of cocaine, an equivalent amount of plasma raised the pressure from 140 to
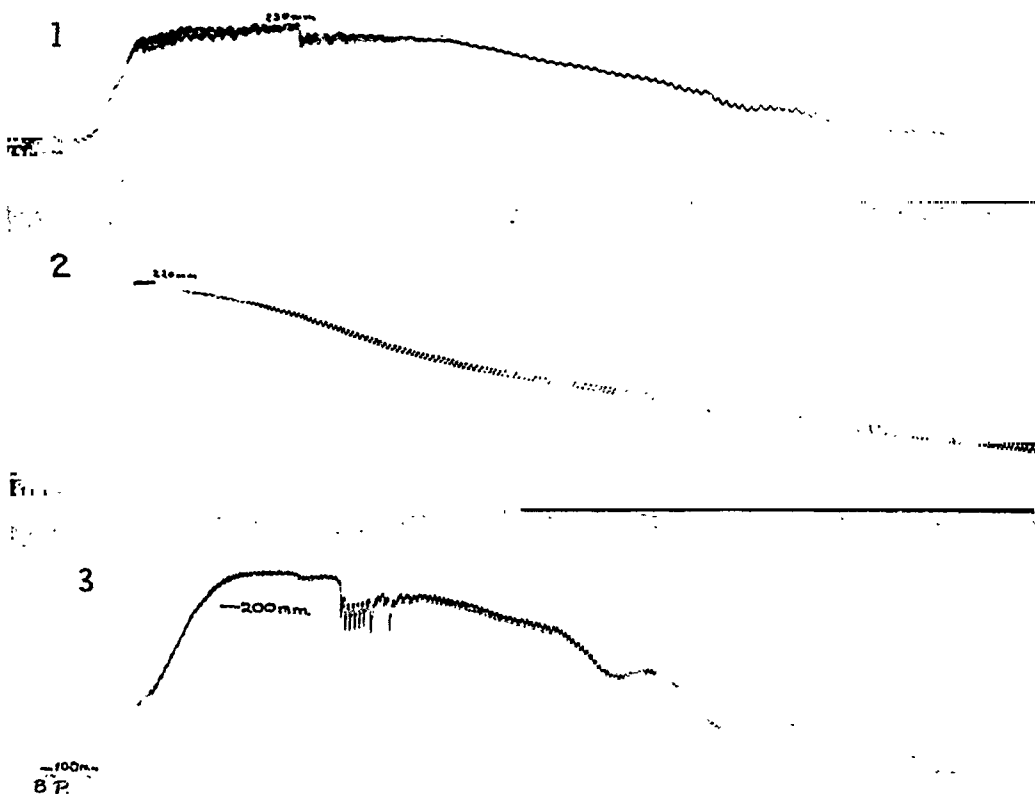
Fig. 1. Shows the effect of the injection of 1 cc. of the protein-free solution made from renal cortical extract anaerobically incubated with dopa. The blood pressure rises from 140 to 230 mm. Hg, the rise lasting three and one-half minutes. (July 9, 1940. Cat ♂, 3.2 kgm., nembutal anesthesia.)

Fig. 2. Shows the effect of the injection of 1 cc. of the same preparation after the injection of cocaine hydrochloride. The potentiating effect of the drug on the height and duration of the rise is noticeable. The blood pressure rises from 80 mm. to 220 mm. Hg, the effect lasting for four and one-half minutes. (Same animal as used in fig. 1.)

Fig. 3. Shows the action of 5 cc. of plasma obtained from kidney perfusate. The blood flow through the perfused kidney was reduced from 15 to 5 cc. per minute. Dopa had been added to the perfusate at the beginning of the perfusion. The blood pressure rises from 100 to 220 mm. Hg, the effect lasting four minutes. (August 20, 1940. Cat ♂, 3.4 kgm., nembutal anesthesia.)

250 mm. Hg. In this case, as in the preceding one, the injection of cocaine prolonged the duration of the rise in pressure.

In a third experiment the renal blood flow was lowered from 15 to 10 cc. per minute. Injection of 5.5 cc. of plasma, obtained by centrifuging the perfusion fluid, evoked only a slight rise in the blood pressure of the cat.

However, its duration was potentiated by cocaine, as in previous experiments.

It can be concluded that the perfused kidney has the capacity which is inherent in extracts of renal cortex to transform dopa into a pressor substance. The amount of this pressor substance produced depends on the blood flow through the kidney. The identity of the pressor substance produced by perfusion of the kidney with dopa dissolved in blood, and that resulting from the action of renal cortical extracts on dopa, is indicated by the fact that in each case oxygen lack is a necessary condition and that the action of the pressor substance produced by each of these two methods is potentiated by cocaine.

Since Holtz claimed (5) that the dopa-decarboxylase, which is necessary for the production of hydroxytyramine from dopa, is found in the liver and in the gut of the guinea pig, experiments were undertaken to determine whether the pressor amine could be produced from dopa by perfusion of the liver or gut of the cat. The liver was perfused through the portal vein at a pressure head of 25/15, the gut at 100/80 mm. Hg. In each case the lung was included in the perfusion circuit.

Perfusion of the liver took place for two hours. The perfusate consisted of 100 cc. of heparinized blood. After constant blood flow through the liver had been established, the rate of venous outflow from the organ was reduced from 60 to 10 cc. per minute. After thirty minutes of perfusion 15 cc. of blood were removed as control, and immediately afterwards 200 mgm. of dopa dissolved in 30 cc. of Ringer's solution were added to the perfusate. After completion of the perfusion 6 cc. of the perfused plasma were injected into a cat and the blood pressure was recorded. No rise was noticeable. The control plasma also failed to evoke a response.

The gut was perfused with 110 cc. of blood for one hour and thirty minutes through the superior mesenteric artery and the rate of blood flow reduced from 20 to 5 cc. per minute. After thirty minutes of perfusion 15 cc. of blood were drawn from the perfusate to serve as a control, and 220 mgm. of dopa were added to the perfusion fluid. After two hours the perfusate was removed and centrifuged. Five and one-half cubic centimeters of perfused plasma, as well as an equal amount of the control serum, injected into a cat, had no effect on the blood pressure.

In three cases it could be shown that blood alone, when incubated with dopa, does not form any of the pressor amine. This was demonstrated in experiments in which 40 cc. of heparinized blood were incubated at 38° with 100 mgm. of dopa under nitrogen for twenty-three hours. After that period, the blood was centrifuged and 5 cc. of the plasma injected. In two experiments a slight rise in the blood pressure was noticeable. However, the injection of cocaine hydrochloride abolished this effect. This indicates that the rise was not due to hydroxytyramine but to some

other pressor substance present in the blood (6).   It was evident, therefore, that the perfused liver, the perfused gut and incubated blood could not transform dopa into hydroxytyramine.   Consequently, of the organs studied, the kidney was the only one to possess this faculty.

DISCUSSION.   Experiments performed on anoxic kidney extracts indicate that under conditions of oxygen-lack, dopa (dihydroxyphenylalanine) is converted into a substance which raises the blood pressure of the cat. Cocaine enhances the rise as well as the duration of the response.   It is the latter, however, which is mainly potentiated.   It seems justified to conclude that the substance described in these experiments is identical with hydroxytyramine.   This assumption is supported by the facts that: 1, Holtz' technique is followed as far as the experiments on renal extracts are concerned; 2, the pressor substance originates only in the presence of dopa and the absence of oxygen; and finally 3, its effect is enhanced by cocaine.

TABLE 1

REDUCTION IN THE RATE OF VENOUS OUTFLOW FROM THE KIDNEY	RISE IN BLOOD PRESSURE
cc./min.	*mm. Hg*
12	120
8	104
7	90
5	30
No reduction	No rise

In investigating the influence of oxygen upon the formation of hydroxytyramine it was found that the yield of pressor substance was decreased under aerobic conditions.   This may be explained by an amino-oxidase which, according to Holtz (1), is found in the renal cortex, and in the presence of oxygen transforms hydroxytyramine into dihydroxyphenylacetaldehyde, a depressor substance.   The fact that some pressor amine was obtained in one case in which the reaction was carried out in the presence of oxygen can be explained by the fact that an excess of dopa was present, and the amino-oxidase was unable to oxidize all the hydroxytyramine which had been formed.   This is confirmed by the finding that when smaller concentrations of dopa were used, no trace of a pressor substance could be detected.

The experiments on the perfused kidney indicate that the intact organ transforms dopa into a pressor substance which is secreted into the perfusion fluid.   This was demonstrated in experiments in which the perfused plasma raised the blood pressure of the cat.   Elevations as high as 120 mm. Hg lasting for eight minutes were obtained.   It can be assumed

that hydroxytyramine is the active agent produced by the perfused kidney, since: 1, cocaine enhances its action, and 2, the presence of dopa and anaerobic conditions are necessary factors in its production.

Reduction of the renal blood flow is essential in the formation of the amine. This is demonstrated in experiments which showed that the reduction of the blood flow through the kidney paralleled the amount of hydroxytyramine present in the perfusate.

Since the pressor amine could not be detected after anaerobic incubation of blood and dopa, the appearance of the pressor amine is exclusively due to an enzyme present in the kidney itself.

Holtz claimed (5) that the liver and the small intestine of guinea pigs contain the dopa-decarboxylase which is the active catalyst in the formation of hydroxytyramine from dopa. Both the liver and the intestine of the cat, perfused with blood containing dopa under conditions of reduced venous outflow, failed to form the pressor amine. Therefore, the perfused cat's kidney is distinguished from the intestine and liver in its ability to form hydroxytyramine.

SUMMARY

1. The production of a pressor substance, presumably hydroxytyramine, by decarboxylation of dopa occurs in extracts of guinea-pig's kidneys under conditions of oxygen lack.

2. A similar reaction takes place in ischemic cat's kidneys perfused with blood containing dopa.

3. The amount of the pressor substance produced in the perfused kidney depends on the rate of flow through that organ.

4. Perfusates of liver and gut of the cat, produced under analogous conditions, as well as cat's blood incubated with dopa, have no pressor properties.

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THE RELATIONSHIPS BETWEEN TOTAL, ACID, AND NEUTRAL CHLORIDES OF GASTRIC JUICE

JOHN S. GRAY, GLADYS R. BUCHER AND HARRY H. HARMAN

From the Department of Physiology and Pharmacology, Northwestern University Medical School and the Department of Education, University of Chicago

A number of theories have been advanced to account for the variation in the composition of gastric juice. Pavlov (1) claimed that gastric juice as it flows from the gastric glands has a constant acidity which may be modified to some extent by admixture with alkaline mucus. Roseman (2) on the other hand maintained that gastric juice as it flows from the gastric glands has a constant total chloride concentration; variations in the acidity of the juice are due to the conversion of a variable proportion of neutral chloride to acid chloride by the parietal cells. Hollander (3) has concluded that gastric juice consists of a variable mixture of a pure isotonic solution of HCl secreted by the parietal cell and an alkaline secretion of constant composition containing all the other elements of the juice. On the other hand, Liu, Yuan and Lim (4) believe that gastric juice consists of a mixture of three components; *a*, a pure hypertonic solution of HCl; *b*, an alkaline, chloride-free fluid secreted at a constant rate, and *c*, a neutral chloride fluid secreted at a rate proportional to that of the acid component.

The above theories are based on observations or assumptions regarding the behavior of the various chloride fractions in gastric juice. The contradictory and incompatible nature of the theories is the result of serious disagreement about these fundamental observations and assumptions. It was hoped, therefore, that a careful study of the variation in the chloride fractions as they are related to one another and to the rate of secretion would permit a choice between the theories or clearly define a new interpretation.

METHODS. Dogs with pouches of the entire stomach (vagotomized) were used in this study. Special measures have been taken to minimize certain sources of error in the collection of gastric juice. The most important innovation consisted in maintaining a continuous and uniform rate of secretion by repeated subcutaneous injections of histamine at ten minute intervals. In order to produce different rates of secretion the dosage of histamine was changed from time to time and from day to day,

so that the scale of volume-rate ranging from 5 to 35 cc. per twenty minutes was covered several times. By this technique the volume of juice secreted in a given period was an accurate measure of the rate of secretion which persisted throughout that period. Samples were collected at twenty minute intervals so that opportunity for variations in secretory rate were still further limited.

Errors due to irregular flow from and temporary retention of juice within the pouch were controlled by two methods. First, the high rates of secretion obtained provided a large enough volume so that regular flow was promoted and the effect of retention of small quantities of juice reduced. Secondly, the device used for the collection of juice assured complete collection without possibility of obstruction of the flow.

In every experiment, samples of gastric juice were not collected for analysis until after a profuse flow of secretion had been stimulated during the first hour and the resulting juice discarded. This flow served completely to flush accumulated mucous secretion from the stomach. This precaution has not been observed by previous experimenters.

Two dogs were used once a week for eight weeks, providing 100 samples of gastric juice from each. In addition 183 samples were collected from six dogs on two successive days and pooled at the time of collection according to the volume-rate of secretion. Nine pooled samples were thus obtained with the average volume-rate of the lowest 6.0 cc. and of the highest 29.1 cc. per twenty minutes. The large number of observations was the final assurance that the errors would be minimal in the final results.

Aliquots of each sample of gastric juice were taken for total chloride determination by the method of Na_2CO_3 fusion followed by Volhard titration and for titration of total acidity, using phenolphthalein as indicator. Neutral chloride was calculated as the difference between total and acid chloride. All analyses were done in duplicate and repeated, if necessary.

Statistical methods were employed in the analysis of the data for two reasons. First, it was essential to know the reliability of the results, and second, the only accurate method of discovering the nature of a relationship which may exist between two variables is to determine by statistical means the line which best fits the plotted points. The usual statistical methods for curve fitting yield two lines, known as regression lines. These are obtained, in principle, by allowing each of the variables in turn to be independent and assuming that the failure of points to fall on the theoretical line is due to variation in the dependent variable only. If the points are widely scattered, the two regression lines may be quite dissimilar. Under such circumstances one may obtain a single, mutual regression line which places equal responsibility on the two variables for failure of the points to fall on the theoretical line. In this investigation, when the ordinary regression lines have given widely differing predictions, mutual

regression lines have also been fitted. In these cases, the obviously divergent predictions have been excluded from the final averages.

For the statistical procedures involved in fitting ordinary regression lines (linear or curvilinear) the principles outlined by Schultz (6) were employed. Since the authors are not aware of any published account of the method of fitting a mutual regression line, the formulas for that purpose are given here.[1] The mutual regression equation may be written:

$$A + BX - Y = 0,$$

where A and B are constants determined by statistical methods and are given by the following formulas:

$$B = -K \pm \sqrt{K^2 + 1}, \qquad A = M_y - BM_x,$$

in which $K = \dfrac{\sigma^2_x - \sigma^2_y}{2\sigma_x \sigma_y r_{xy}}$ and M_x, M_y are the means of the respective variables, the σ's their standard deviations, and r_{xy} their correlation coefficient. There are two values for B—one gives the best fitting line and the other the worst—but the value to be taken in any practical case is obvious from the nature of the data. The standard errors of the constants A and B are not known, but the standard error of estimate, S, in predicting either X or Y may be obtained from the formula,

$$S^2 = \tfrac{1}{2}(\sigma^2_x + \sigma^2_y) - \tfrac{1}{2}\sqrt{(\sigma^2_x - \sigma^2_y)^2 + 4\sigma^2_x \sigma^2_y r^2_{xy}}$$

RESULTS. *Volume-output method.* When the output of either total chloride, acid chloride, or neutral chloride is plotted against the volume-rate of secretion, a linear relationship immediately becomes apparent. This is shown in figure 1, in which the raw data obtained from the pooled samples are plotted together with the statistically-fitted regression lines. The statistical data, including correlation coefficients, the constants in the regression equations, standard errors of these constants, and the standard errors of estimate are presented in table 1. The correlation coefficients are remarkably high.[2] The standard error of the b constant, or slope, is very small in each case. With the exception of the equations relating the output of neutral chloride to the volume-rate of secretion, the two ordinary regression equations yield almost identical predictions.

The important fact to be noted is that as *the volume-rate of secretion increases, the outputs of total chloride, acid chloride, and neutral chloride all*

[1] These formulas were taken from a series of lectures by Professor Schultz at the University of Chicago.

[2] This is due to the repetition of the variable, volume-rate, i.e., the output figures were all obtained by multiplying concentration by volume-rate, and this product was then correlated with the volume-rate. This spurious correlation, however, only augments a pre-existing correlation.
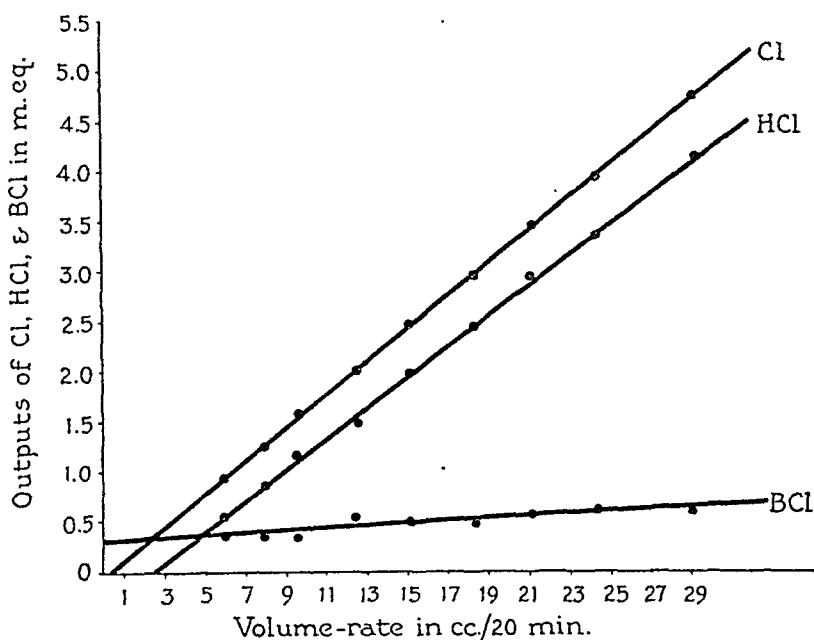
Fig. 1. Relationships between the volume-rate of secretion and the outputs of the chloride fractions for the "pooled samples." (Volume-output method.)

TABLE 1

Linear relationships between volume-rate and outputs of chloride fractions
(Volume-output method)

	DOG 1			DOG 2			POOLED SAMPLES		
	Cl	HCl	BCl	Cl	HCl	BCl	Cl	HCl	BCl
	Correlation coefficient								
	0.999	0.996	0.541	0.999	0.997	0.783	0.999	0.999	0.928
	Volume independent*								
a	−0.0715	−0.2613	0.1899	−0.0632	−0.2118	0.1486	−0.0527	−0.3550	0.3009
b	0.1667	0.1575	0.00921	0.1657	0.1518	0.0139	0.1649	0.1535	0.0115
σ_b	0.00086	0.00105	0.00166	0.00037	0.00078	0.00112	0.00088	0.00142	0.00175
S_x	0.0455	0.0554	0.0880	0.0352	0.0699	0.0752	0.0172	0.0277	0.0341
	Volume dependent†								
a'	0.4645	1.1766	−14.28	0.3976	1.4756	−22.60	0.3336	2.1800	−23.13
b'	5.9835	6.2946	31.92	6.0293	6.5529	44.10	6.0590	6.5780	80.73
$\sigma_{b'}$	0.0308	0.0419	5.01	0.0136	0.0329	3.54	0.0324	0.0610	12.29
S_v	0.272	0.350	4.49	0.213	0.459	5.59	0.104	0.181	2.76

* The regression of Cl, HCl, or BCl (designated by x) on volume-rate (V) has the following form:

$$X = a - bV.$$

The standard error of estimate of X is denoted by S_x and the standard error of b, the slope of the line, by σ_b.
  † The regression of V on X is given by $V = a' + b'X$, and the standard errors are designated similarly to the preceding case.

increase in a linear fashion, but at different rates. The slope of the total chloride line is significantly greater than that of the acid chloride line, and hence the two lines are divergent; consistent with this is the fact that the output of neutral chloride increases with the volume-rate of secretion.

From the volume-output equations one can predict the relationship existing between the rate of secretion and the concentration of the chloride fractions of the gastric juice. For example, the equation relating the total chloride output to the volume-rate has the following form:

$$Cl = a + bV.$$

By dividing this equation by volume-rate, V, we obtain, $\dfrac{Cl}{V} = \dfrac{a}{V} + b$, in which $\dfrac{Cl}{V}$ represents the *concentration* of total chloride. This equation has the form of a hyperbola, in which the constant, b, represents the asymptotic value. This asymptote reveals the maximal concentration of total chloride which the gastric juice can attain. The same procedure can be applied to the equations for acid chloride and neutral chloride outputs. Predictions made by this method for the maximal concentrations of total and acid chlorides and the minimal concentration of neutral chloride will be found in table 4. From this table one can also see the close agreement between the predictions from the two ordinary regression lines.

Since algebraic manipulations of regression equations may introduce further inaccuracies in predictions, it is desirable to investigate *directly* the relationship between the volume-rate of secretion and the concentrations of the chloride fractions. The above discussion, however, serves to indicate that the relationships are hyperbolic in form and that hyperbolic regression equations should be fitted to the data.

Volume-concentration method. When the concentrations of total chloride, acid chloride, or neutral chloride are plotted against the volume-rate of secretion, hyperbolic relationships become evident. This is shown in figure 2, in which the raw data obtained from the pooled samples are plotted together with the statistically-fitted regression curves. The correlation indexes and other statistical parameters are presented in table 2. The correlation indexes are lower than the correlation coefficients of the previous method; since the volume-rate and the concentrations of the chloride fractions were determined independently, spurious correlation is not present. Only one hyperbolic regression curve has been fitted to each set of observations.[3]

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[3] Since the observations are spread out along the horizontal part of the curve, only the regression equations which are obtained by minimizing the deviations in the vertical direction are of significance in this case.

It should be noted that *as the volume-rate of secretion increases, the concentrations of total chloride and acid chloride increase in a hyperbolic fashion,*
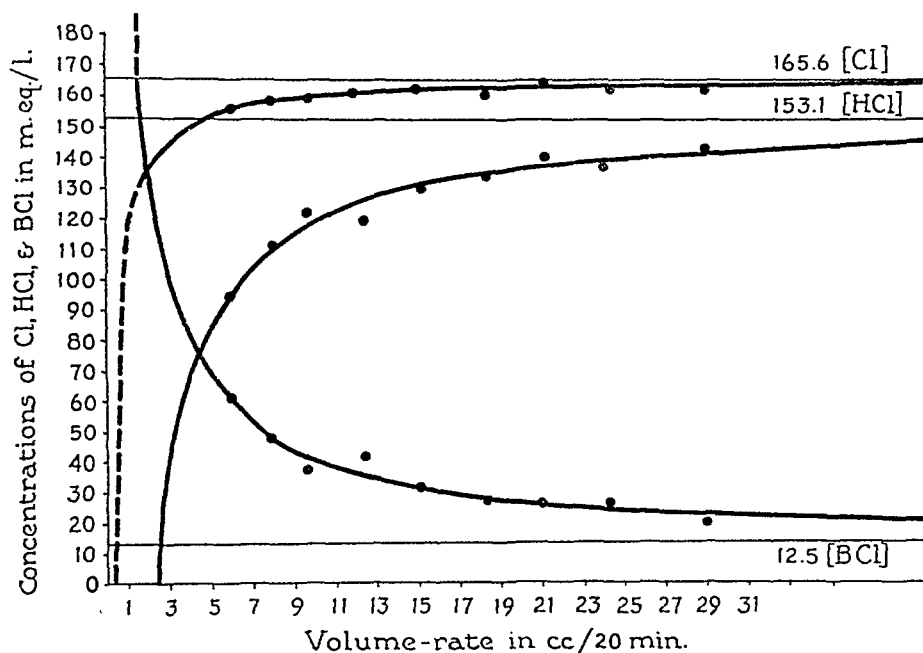
Fig. 2. Relationship between the volume-rate of secretion and the concentrations of the chloride fractions for the "pooled samples." (Volume-concentration method).

TABLE 2

Relationships between the volume-rate and the concentration of the chloride fraction

(Volume-concentration method)
Volume independent*

	DOG 1			DOG 2			POOLED SAMPLES		
	[Cl]	[HCl]	[BCl]	[Cl]	[HCl]	[BCl]	[Cl]	[HCl]	[BCl]
	Correlation index								
	0.522	0.696	0.590	0.554	0.779	0.727	0.955	0.987	0.978
c	−63.99	−230.96	166.97	−40.61	−180.76	140.15	−62.98	−349.36	286.38
d	166.09	155.19	10.90	164.04	149.71	14.33	165.57	153.05	12.52
σ_d	0.90	2.04	1.95	0.58	1.38	1.26	0.72	1.93	2.06
$S_{[x]}$	2.99	6.79	6.52	2.83	6.74	6.13	0.88	2.36	2.52

* The hyperbolic regression of [Cl], [HCl], or [BCl], designated by [x], on V is written in the form:

$$[x] = \frac{c}{V} + d.$$

whereas the concentration of neutral chloride decreases in a hyperbolic fashion. Since these relationships are hyperbolic in form, the asymptotes, or *b* con-

stants, reveal the limiting concentrations of the chloride fractions of gastric juice. The maximal concentration of total chloride varies from 164.0 to 166.1 milli-equivalents per liter. The maximal concentration of acid chloride varies from 149.7 to 155.2 milli-equivalents per liter. The minimal concentration of neutral chloride varies from 10.9 to 14.3 milli-equivalents per liter. The consistency of the results is shown by the fact that the limiting values for these elements are not significantly different from dog to dog. The difference between the maximal total chloride and acid chloride concentration is statistically different in each case. *The fact that the minimal concentration of neutral chloride in each case is positive and significantly[4] different from zero proves that even at infinitely high rates of secretion neutral chloride is present in the gastric juice.*

A remarkably close agreement exists between the limiting values determined directly by the volume-concentration method and those indirectly predicted from the volume-output method. This comparison can best be made by consulting the first three columns of table 4.

Another important relationship is evident from figure 2. The total chloride and neutral chloride lines cross at the point where the acidity of the juice becomes zero. This of course means that in the absence of acid all the chloride is present in the form of neutral chloride. Upon further extrapolation to the left (fig. 2) the neutral chloride line crosses and extends above the total chloride asymptote, implying hypertonic gastric juice; similarly the total chloride line takes on negative values. Obviously extrapolation in this direction leads to conclusions which are absurd from the standpoint of physiology, although Liu, Yuan and Lim based their theory on this unwarranted procedure.

Concentration-concentration method. When the concentration of either total chloride or neutral chloride is plotted against the acidity of gastric juice, a linear relationship becomes apparent. This is shown in figure 3, in which the raw data obtained from the pooled samples are plotted together with the statistically fitted mutual regression lines. The statistical results are presented in table 3. In relating the acidity to the neutral chloride concentration higher[5] correlation coefficients were obtained than in relating the acidity to the total chloride concentration. In cases where the correlation coefficients were low, predictions from the two regression equations differed considerably, hence mutual regression lines were also fitted to the data.

It should be noted that *the acidity of gastric juice varies directly with the*

[4] The minimal concentration of neutral chloride is more than three times its standard error, or more than approximately 4.5 times its probable error.

[5] This is again due to spurious correlation resulting from the fact that the neutral chloride concentrations were determined by subtracting the acid chloride from the total chloride.

total chloride concentration and inversely with the neutral chloride concentration. From the equations representing these relationships one can calculate the maximal total or acid chloride concentration at the point where neutral chloride is assumed to be absent. Taking only the more reliable estimates, the maximal acid or total chloride concentration obtained by this method varies from 163 to 168 milli-equivalents per liter, as shown in table 4. *By this method the maximal acidity of gastric juice is found to be significantly higher than by the volume-concentration method. This discrepancy results from the assumption that neutral chloride can be absent from gastric juice.*
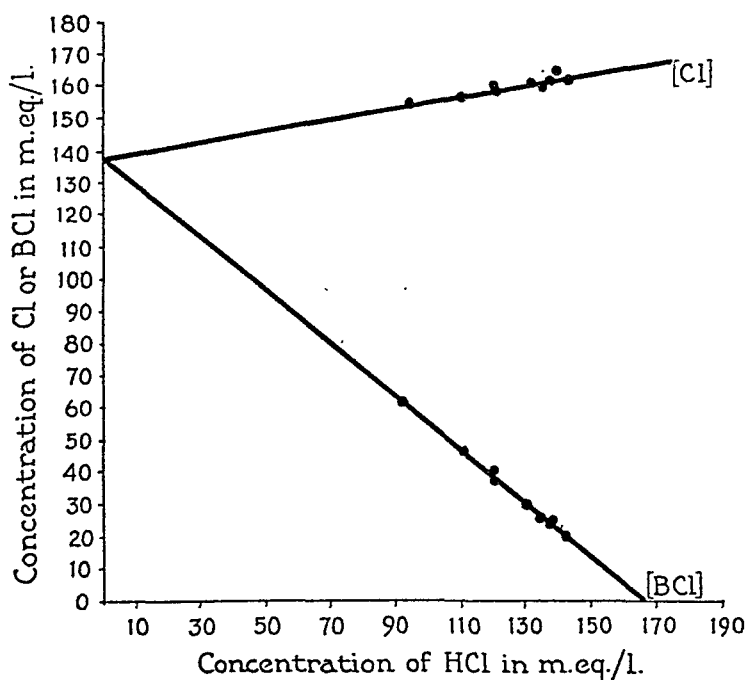
Fig. 3. Relationships between the concentration of the chloride fractions for the "pooled samples". (Concentration-concentration method.)

DISCUSSION. It has been claimed (2) that the total chloride concentration of gastric juice is constant, but the results obtained by ourselves as well as others (3, 4, 7) demonstrate very definitely that it varies both with the rate of secretion and the acidity. This finding is opposed to the theory of Rosemann for the variations in the composition of gastric juice. This behavior of the total chloride has been frequently overlooked because the range of variation is small and noticeable only at the slower rates of secretion.

Pavlov (1) claimed that if accumulated mucus is first flushed from a gastric pouch, the acidity remains constant. Hollander (3) has reported

the same if the juice is collected in such a way as to prevent irritation and consequent mucus production.   On the other hand we have found that

TABLE 3

Linear relationships between the concentration of the chloride fraction

(Concentration-concentration method)

	DOG 1		DOG 2		POOLED SAMPLES	
	[Cl]	[BCl]	[Cl]	[BCl]	[Cl]	[BCl]
	Correlation coefficient					
	0.552	−0.932	0.647	−0.957	0.871	−0.993
[HCl] independent*						
a	132.95	132.93	133.10	133.08	138.43	138.41
b	0.2050	−0.7948	0.2047	−0.7952	0.1766	−0.8232
σ_b	0.0312	0.0313	0.0244	0.0243	0.0375	0.371
$S_{[x]}$	2.92	2.93	2.59	2.59	1.45	1.44
[HCl] dependent†						
a'	−102.69	163.23	−193.78	164.64	−564.66	167.53
b'	1.4876	−1.0923	2.0460	−1.1520	4.2966	−1.1976
σ_b'	0.227	0.0429	0.244	0.0353	0.916	0.0539
$S_{[HCl]}$	7.88	3.43	8.19	3.12	2.42	1.73
Mutual regression‡						
A	130.16	139.04	131.46	136.94	138.22	139.05
B	0.2254	−0.8468	0.2168	−0.8238	0.1783	−0.8282
C	2.86	2.21	2.54	2.01	1.45	1.10

* The regression equation has the form:

$$[x] = a + b[HCl],$$

where $[x]$ stands for $[Cl]$ or $[HCl]$.   The remaining symbols are used as in previous tables.

† The regression equation with $[HCl]$ as dependent variable is written as follows:

$$[HCl] = a' + b'[x].$$

‡ The symbols employed in the mutual regression equations are the same as those in the equations under methods with $x = [HCl]$ and $y = [Cl]$ or $[BCl]$.   For example, the first equation can be written out as follows:

$$130.16 + 0.2254[HCl] − [Cl] = 0.$$

the acidity varies with the rate of secretion, even when accumulated mucus has been flushed from the pouch.   The discrepancy is only superficial, for the hyperbolic form of the acidity curve permits wide fluctuations in high rates of secretion without significant changes in acidity (fig. 2).   If

TABLE 4

Summary of limiting values for chloride fraction

| | VOLUME-OUTPUT METHOD | | VOL.-CONC. METHOD | CONCENTRATION-CONCENTRATION METHOD | | | | | |
| | | | | [HCl]-[Cl] relationship | | | [HCl]-[BCl] relationship | | |
	Volume independ.	Volume depend.	Volume independ.	[HCl] independ.	[HCl] depend.	Mutual	[HCl] independ.	[HCl] depend.	Mutual
Maximal [Cl]									
Dog 1	166.7 ±0.86	167.2 ±0.87	166.1 ±0.90	167.2±2.92	210.6±7.88	168.0±2.86	167.2±2.93	163.2±3.43	164.2±2.21
Dog 2	165.7 ±0.37	165.8 ±0.37	164.0 ±0.58	167.3±2.59	185.3±8.19	167.9±2.54	167.3±2.59	164.6±3.12	166.2±2.01
Pooled samples	164.9 ±0.88	165.0 ±0.88	165.6 ±0.72	168.1±1.45	171.3±2.42	168.2±1.45	168.1±1.44	167.5±1.73	168.0±1.10
Maximal [HCl]									
Dog 1	157.5 ±1.05	158.9 ±1.06	155.2 ±2.04	Same as above					
Dog 2	151.8 ±0.78	152.7 ±0.77	149.7 ±1.38						
Pooled samples	153.5 ±1.42	152.0 ±1.41	153.1 ±1.93						
Minimal [BCl]									
Dog 1	9.17±1.66	31.35±4.92	10.90±1.95	0	0	0	0	0	0
Dog 2	13.9 ±1.12	22.68±1.82	14.33±1.26	0	0	0	0	0	0
Pooled samples	11.49±1.75	12.39±1.89	12.52±2.06	0	0	0	0	0	0

mucus production is minimized, the secretion rates must obviously be still lower before they affect the acidity.

By the two "volume" methods the maximal total chloride concentration averaged 165.7 m.eq./l, a value which is isotonic as would be demanded by the observations of Gilman and Cowgill (9). By similar methods Liu, Yuan and Lim obtained 176 for the dog and Wang 158.6 for man. The high and probably incorrect value of 176 may be due to the state of the dogs or to faulty methods for determining total chloride, but the other possibilities suggested by Hollander (8) do not apply. There appears to be no significant discrepancy between the various estimates for maximal acidity obtained by the "volume" methods (table 5). Of most significance is our confirmation of Liu et al., and Wang that the neutral chloride concentration does not fall to zero. Neither we nor any other investigators have observed samples of gastric juice free of neutral chloride; these results show that even at infinitely rapid rates of secretion neutral chloride will still be present.

TABLE 5

Final average values

All values as milli-equivalents per liter

| | VOLUME METHODS | | | CONCENTRATION-CONC. METHOD | |
	Authors' Dog	Liu et al. Dog	Wang Man	Authors' Dog	Hollander Dog
Maximum total chloride conc.....	165.7	176.0	158.6	166.9	165.2
Maximum acidity................	153.8	157.7	139.5	166.9	165.2
Minimal neutral chlor. conc......	12.1	18.3	26.8	0	0
Osmotic press. of blood..........		168	159		

Of the several theories which have been advanced to explain the variations in the composition of gastric juice, none seems to fulfill the requirements set by the available observations. The insurmountable objection to Rosemann's theory has been mentioned. The theory of Liu, Yuan and Lim, which assumes that the gastric glands secrete a chloride-free fluid, was based on the unjustified extrapolation of the chloride hyperbola pointed out under Results.

Hollander's theory is also based on an extrapolation that is questionable. He assumed that the parietal cell secretes no neutral chloride and justified this assumption by demonstrating that its use led to an acceptable value for the acidity of the parietal secretion. From the straight line relating the concentrations of acid and neutral chloride, Hollander found that the intercept corresponding to zero concentration of neutral chloride represented 165.2 m.eq./l. of acid. The same procedure applied to our data (concentration-concentration method) yields 166.9 m.eq./l.

It should be noted, however, that this figure is significantly greater than the maximal acidity obtained from the acidity hyperbola and slightly, but uniformly, greater than the maximal total chloride concentration obtained similarly.   This is, of course, the inevitable consequence of assuming the neutral chloride concentration to be zero, as Liu, Yuan and Lim insisted in their rejection of Hollander's method.

The objection, therefore, to Hollander's theory is that it maintains that neutral chloride can be absent from gastric juice, which is contrary to all observation.

Before one can advance any theory to account for the variations in the composition of gastric juice, a fundamental question must be answered. *Why is neutral chloride always present in gastric juice?*   Is it because the parietal cells secrete neutral chloride, or, is it because an increase in the activity of the parietal cells is accompanied by an increased secretion of neutral chloride by non parietal cells?   Since the present analysis of the behavior of only the chloride fractions provides no answer to this question, no theory can as yet be constructed to account for the composition of gastric juice.

CONCLUSIONS

1. The relationships between the various chloride fractions of gastric juice have been investigated by a statistical analysis of data obtained from 383 samples of gastric juice carefully collected from dogs with vagotomized pouches of the entire stomach.   The continuous secretion of gastric juice at various rates was maintained by subcutaneous injections of histamine at ten minute intervals.

2. As the volume-rate of secretion increases, the *outputs* of total, acid, and neutral chlorides all increase in a linear fashion, but at different rates.

3. As the volume-rate of secretion increases, the *concentrations* of total and acid chlorides increase in a hyperbolic fashion, whereas the concentration of neutral chloride decreases in a hyperbolic fashion.

4. As the acidity of gastric juice increases, the total chloride concentration increases and the neutral chloride concentration decreases, both in a linear fashion.

5. From the statistically fitted equations representing the above relationships it has been found that at infinite rates of secretion the maximal total chloride concentration of gastric juice is 166 milli-equivalents per liter, the maximal acidity, 154 milli-equivalents per liter, and the minimal neutral chloride concentration, 12 milli-equivalents per liter.

6. None of the various theories advanced to explain the variations in the composition of gastric juice are wholly acceptable in the light of the above findings.

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OBSERVATIONS ON THE WATER INTAKE IN AN ADULT MAN WITH DYSFUNCTIONING SALIVARY GLANDS[1]

F. R. STEGGERDA

From the Department of Physiology, University of Illinois, Urbana

The theory of Cannon (1) that the salivary glands are essential to thirst and control of water uptake in man has been questioned in recent years by different investigators (2), (3), (4), (5). Dill (6) in a critical review of the literature concludes that the original stimulus of thirst probably is located in the muscle cells and is brought about when an upset in the electrolytic balance occurs. Although the experimental evidence opposing Cannon's view may be correct and justified, it should be pointed out that in these recent studies comparisons are being made between results obtained from normal human beings and from animals which, in many cases, had previously been prepared by operation for such experimental purposes. It is also true that the experiments of Cannon on man were carried out over relatively short periods of time and under rather acute conditions, while the work on animals in many cases was made several days or months after the animal had become adjusted to his new environmental state. For obvious reasons many of the experiments carried out on animals could hardly be duplicated on man, although it would be the ideal way to make accurate comparisons.

It was our good fortune to come upon a University student who claims that he has been without salivary secretions since earliest infancy. A medical examination confirmed a complete absence of all salivary glands and ducts. Likewise, no change in mouth secretions was recorded following subcutaneous injections of 6.0 mgm. of pilocarpin. Although the mucous membranes of the mouth were always dry, there was evidence of a certain amount of mucinous secretion from gland cells along the inside of the cheeks and under the tongue. Upon testing these secretions with starch paste, there was no evidence of the presence of amylolytic enzymes (7).

The subject also reports a deficiency of lachrymal secretion, as well as the early onset of dental caries, so severe that false dentures were needed

at the age of 21 years. Similarly, the recent experiments of Cheyme (8) show that rats without salivary glands show a greater frequency of dental caries. His dry mouth condition does not interfere with his sleep. Adolph (4) has also observed that the sleep of dogs with esophageal fistulae is not interrupted if the water deficiency is of a mild degree.

The subject has learned to relieve the dry uncomfortable feeling in his mouth by taking a few swallows of water nearly every hour. Although at times he can go without water for two hours, this occurs very infrequently. Along with the frequent intake of small quantities (60 cc.) at approximately hourly intervals, he becomes actually thirsty about four times a day when his consumption is about 250 cc. per draft. A simple calculation of the total water intake per day, excluding water in the food, would give him 1960 cc. assuming that he drinks small quantities of 60 cc. on 16 hourly occasions. This calculated water intake agrees closely with previously reported experiments (9) in which a comparative study of the total fluid intake of this subject and four normal persons was made over a period of 18 days. The average daily intake for the four control subjects was 1740 cc., while the total fluid intake, which included coffee, tea and milk, gave an average figure of 2615 cc. During the same period, the experimental subject showed such average quantities as 1975 and 2783 cc. respectively. Since Richter (10) has recently reported that the amount of fluid intake in man and animals is related to body size, we have recalculated our data on the basis of surface area and found the amounts to vary from 1,145 to 1,476 cc. per square meter, with the exception of one subject in the control group, who habitually drank extremely large quantities of tea and coffee each day, the subject without salivary glands consuming 1,421 cc. per sq.m. of surface area. Judging from these experiments it would seem that the total daily fluid intake is not materially affected by the lack of salivary glands as registered by the experimental subject.

It is a well known fact that a normal person with a dry mouth has a pronounced desire to drink. Therefore, we decided to induce this type of thirst in normal individuals in order to determine the amount of fluid they would require to relieve their discomfort, and then compare their intake with that of the experimental subject. Nine normal individuals were allowed to drink as much water as they desired to satisfy their thirst, and then, immediately after, the mucous membranes of their mouths were exposed to a stream of warm, dry air until they became very thirsty. Water was supplied in calibrated containers and the subjects were instructed to drink until their thirst was relieved. It was interesting to note that the average intake for the nine subjects was 88 cc. Later experiments, in which the subjects were instructed to drink slowly and only just enough to satisfy their thirst, gave practically the same results. On the basis of

60 cc. per draft in the experimental subject, this was 47 per cent greater. In no case did the mucous membranes of the mouths of the controls appear as dry as those of the experimental subject.

To find whether there was any possible difference in body water metabolism levels between the experimental and normal subjects, experiments were made in which they were given rather large quantities of sodium chloride by mouth, after which a record was made of water intake, urine production, and the amount of salt excreted in the urine. This, according to Coller and Maddock (11), would give us information concerning the physiological normalcy of the experimental subject; also it would show whether an actual desire or thirst for water could be elicited in him.

The experimental procedure was as follows: on five different occasions the experimental subject and two controls were given 10 grams of sodium chloride in 100 cc. of water by mouth, about 12 to 14 hours after their last meal. Hourly records of water intake, urine output, and excretion of urine chlorides (Volhard-Harvey method) were made for a period of six hours. We chose this length of time because Adolph (4) had reported that 2 to 5 hours are necessary to make body adjustments following a water deficit in man. No restrictions or record of water intake were made up to the time of the experiment. Each subject reported a definite sensation of thirst immediately following the ingestion of the salt solution and responded by drinking rather large quantities of water. In the first three of the five experiments performed (period I, table 1), in which the subjects were allowed to drink ad libitum, it was noted that the controls drank 42 per cent more water than the experimental subject and also excreted 51 per cent more urine during the time of the experiment. Adolph (4) also reports that dogs, made deficient in water, exceed the expected water intake by 17 per cent when allowed to drink freely. In the last two experiments (period II, table 1) the experimental subject served as a control for the frequency of drinking. Thus, whenever he expressed the desire to drink the two controls were requested to do likewise. It is interesting to note that in this type of experiment the controls again drank more water and excreted more urine than the experimental subject. The actual increase in water intake was 46 per cent and of urine excretion 35 per cent. Table 1 shows that the water intake and urine output were higher in period II than in period I in both control and experimental subjects. This was probably due to the fact that the atmospheric temperature was higher than when the former experiments were performed. The average amounts in both the water intake and the urine output of the controls in the two periods were calculated to be 44 per cent and 42 per cent above those of the experimental subject, but it is noteworthy that the amounts of chloride excreted were the same for all three individuals.

The observation that the water intake in the control subjects was 44

per cent above the experimental subject indicates that the normal individual habitually drinks more water than his body requires. Further, since the ratio between water intake and the urine excretion of the experimental subject is practically the same as that of the controls, and since the amounts of salt excreted are the same, we feel justified in drawing the conclusion that our subject, with the exception of not possessing salivary glands, is physiologically normal.

TABLE 1

The effects of NaCl ingestion on water intake and urine and salt excretion

	TWO CONTROL SUBJECTS				EXPERIMENTAL SUBJECT			
	Time	Water intake	Urine output	Total Cl in urine	Time	Water intake	Urine output	Total Cl in urine
		cc.	*cc.*	*grams*		*cc.*	*cc.*	*grams*
Period I. Average of 3 experiments	1 hr.	323	59	0.378	1 hr.	247	39	0.486
	2 hr.	258	65	0.889	2 hr.	263	64	0.979
	3 hr.	168	81	1.393	3 hr.	102	77	1.203
	4 hr.	190	84	1.000	4 hr.	98	62	0.979
	5 hr.	98	87	0.809	5 hr.	72	46	0.752
	6 hr.	198	119	0.875	6 hr.	88	39	0.687
	Total	1,235	495	5.344		870	327	5.086
Period II. Average of 2 experiments	1 hr.	633	44	0.409	1 hr.	593	36	0.582
	2 hr.	214	59	0.784	2 hr.	198	69	1.041
	3 hr.	384	78	1.177	3 hr.	168	73	1.239
	4 hr.	233	93	1.183	4 hr.	100	75	1.280
	5 hr.	188	112	0.891	5 hr.	80	73	1.230
	6 hr.	173	146	0.816	6 hr.	110	68	1.222
	Total	1,825	532	5.260		1,249	394	6.594
Average of 5 experiments		1,530	514	5.302		1,060	361	5.840

We realize that our findings have not solved the problem of the true site of the thirst sensation, nor do we venture to advance any new theory concerning it. However, we believe that our observations on an adult man without salivary glands will help to bridge a gap between the different types of experiments conducted by previous investigators. Our results, as well as those reported by others on dogs (2), (3), (4), (5), show quite conclusively that the salivary glands, while essential in keeping the mouth moist and comfortable, are not the sole factors governing thirst. Furthermore, we have noted that in all cases where thirst was induced either by drying of the mouth or by the ingestion of sodium chloride, the normal individuals consumed at least 44 per cent or more water than the experi-

mental subject.   Although this may be related to the presence or absence of salivary glands it would seem more logical to explain this on the basis of the drinking habits of the normal individual.   This extra flushing of the body probably has a salutary effect; but it is evident that through control the water intake can be greatly reduced without upsetting the normal functioning level of water in the body..

CONCLUSIONS

1. The absence of salivary glands in man does not cause him to drink greater quantities of fluid than a normal individual.

2. Normal subjects, when made thirsty by artificial means such as exposure of the mouth to dry heat or ingestion of sodium chloride by mouth, consume from 42 to 47 per cent more water to moisten the mouth than the experimental subject habitually drinks.

3. A person without salivary glands may have a normal water and salt metabolism.

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THE ACTION OF DESOXYCORTICOSTERONE ACETATE AND PROGESTERONE ON THE BLOOD AND TISSUE CHLORIDES OF NORMAL AND ADRENALECTOMIZED ANIMALS

HANS SELYE AND CHRISTIANE DOSNE

From the Department of Anatomy, McGill University, Montreal, Canada

In a short note we recently reported (1) that desoxycorticosterone acetate (D.C.A.) given in relatively high doses decreases the blood chloride concentration in the rat. In adrenalectomized animals whose blood chlorides are below normal, D.C.A. exerts the opposite effect. Continuing our studies of this phenomenon we wished to establish whether the same dose of D.C.A. which raises the blood chlorides in the adrenalectomized animal can lower them in the presence of the adrenals. This appeared to be important in order to determine whether the latter action is merely the result of overdosage, obtainable both in the presence and in the absence of the adrenals as long as sufficiently large doses are given, or whether the hypochloremic action is dependent upon the presence of functional adrenal tissue. We hoped furthermore that urine and tissue chloride determinations would help us to understand the mechanism through which D.C.A. decreases the blood chloride concentration in the intact rat. At the same time we wished to investigate the effect of progesterone on the blood chlorides of the adrenalectomized rat since this compound differs from D.C.A. only in that it has no —OH group on C atom 21 and resembles the latter in its pharmacological actions. Both compounds maintain adrenalectomized animals alive, cause progestational proliferation of the endometrium and development of the mammary glands. Yet unlike D.C.A., progesterone does not produce any significant decrease in the blood chloride concentration of the intact rat (2). In view of these observations, additional comparative studies concerning the pharmacological actions of progesterone and D.C.A. appeared necessary to obtain further information concerning the physiological significance of the C 21 hydroxyl group.

METHODS AND MATERIALS. Our first experiments were performed on young adult male and female albino rats, the various groups having an average body weight of 140 to 174 grams. The females were somewhat lighter than the males in most cases. Later we noted, however, that still younger rats are even more suitable for such experiments since they show more rapidly developing and pronounced signs of adrenal insufficiency.

Therefore rats weighing only 80 to 84 grams on the average were used exclusively in our second experimental series.

The adrenalectomies were performed from the dorsal route under ether anesthesia. In the first two experimental series (tables 1 and 2) progesterone or D.C.A. was administered in doses of 5 mgm. twice daily subcutaneously in 0.2 ml. of peanut oil. The injections were started on the day of operation and continued for two days, the last injection being given 6 hours before sacrificing the animals. The slightly different method of treatment used in the last series (table 3) is described in the experimental section. Before every chemical determination, food was withdrawn for 24 hours so as to avoid post-absorptive changes. The animals were killed without anesthesia by cutting the carotid artery and jugular vein.

TABLE 1

Blood and tissue chlorides in adult rats

TREATMENT	WHOLE BLOOD	P	PLASMA	P	MUSCLE	P	BODY WEIGHT	SEX
Intact controls	419		557		135		156	Female
Intact D.C.A.-treated	378	<0.01	572	0.2	146	0.5	154	Female
Adrenalectomized	381	<0.01	563	0.5	160	<0.01	140	Female
Adrenalectomized D.C.A.-treated	397	0.2	561	0.6	189	<0.01	154	Female
Adrenalectomized progesterone-treated	382	0.7	534	0.05	150	0.4	148	Female
Intact controls	407		562		134		157	Male
Intact D.C.A.-treated	378	0.05	556	0.5	138	0.5	174	Male
Adrenalectomized	370	0.05	520	<0.01	135	0.5	155	Male
Adrenalectomized D.C.A.-treated	383	0.1	581	<0.01	193	<0.01	161	Male
Adrenalectomized progesterone-treated	365	0.6	487	0.5	152	0.3	170	Male

All the blood was collected in crucibles using potassium oxalate to prevent clotting. The amount of blood thus collected was accurately measured in the second experimental series (see table 2) because it gives a good indication of the volume of freely circulating blood. Accurate total blood volume determinations are not practicable in animals of this size without perfusion of the vascular system and this would interfere with the blood and tissue analyses. It must be kept in mind however, that the blood volume thus determined is considerably below the total blood volume because much of the blood which stagnates in capillaries and small veins is not recovered.

The tissue chlorides are expressed as milligrams of NaCl/100 ml. of blood or 100 grams of tissue. They were determined on 1 gram samples

by the Van Slyke (3) method and the whole blood and plasma chlorides by a modification of the above technique in which the concentration of the digestion mixture was adjusted for micro-determination on 0.2 ml. samples. The blood sugars are expressed as mgm/100 ml. and were determined by the Somogyi (4) modification of the Shaffer-Hartmann technic.

The significance of the apparent differences between the treated and control groups was evaluated by "Student's" method for small samples (5) and is expressed in the tables in terms of probability, estimated by graphic interpolation in Fisher's table of t. In accordance with the usual convention, differences between series cannot be accepted as significant when P is greater than 0.05. In calculating the significance of the apparent changes the intact untreated animals served as controls for the

TABLE 2

Blood and tissue chlorides, blood glucose and blood volume in young male rats

TREATMENT	WHOLE BLOOD CHLORIDES	P	PLASMA CHLORIDES	P	MUSCLE CHLORIDES	P	BLOOD VOLUME	P	BLOOD GLUCOSE	P	BODY WEIGHT
Intact controls.	416		560		138		1.5		84		80
Intact D.C.A.-treated......	357	<0.01	522	<0.01	155	0.05	1.6	0.7	57	<0.01	81
Adrenalecto-mized........	379	<0.01	507	<0.01	142	0.6	0.8	<0.01	43	<0.01	84
Adrenalecto-mized D.C.A.-treated......	417	<0.01	553	<0.01	169	<0.01	1.4	<0.01	61	0.02	81
Adrenalecto-mized proges-terone-treated......	386	0.5	516	0.5	139	0.5	0.8	0.6	44	0.6	83

intact D.C.A. injected groups while the determinations on adrenalectomized-treated animals were compared with those on untreated adrenalectomized controls.

EXPERIMENTAL RESULTS. The results of our experiments are summarized in the following tables which are self-explanatory and do not require much comment. Each figure represents the average of six individual determinations on six different animals. The results in table 2 are a repetition and confirmation on younger animals of the chloride values given in table 1. In this second experiment, additional data concerning blood volume and blood glucose changes have also been recorded.

As the tables indicate, the whole blood chlorides are decreased after adrenalectomy and restored towards or to normal by D.C.A. but they

remain unaffected by progesterone. The plasma chloride values run approximately parallel to the whole blood chlorides except in the adult females (table 1) in which the average plasma chloride concentration was not below normal either in the case of D.C.A. or adrenalectomy. This might perhaps be ascribed to the fact that the intact controls of this particular group showed an unusually low normal whole blood and plasma chloride concentration for females. As we pointed out in a previous publication (2) usually the normal blood chlorides of females are significantly higher than those of males. In any case the plasma chloride variations, though essentially similar to those of the whole blood, were always much less pronounced. This is in agreement with the view that the hypochloremia produced by adrenalectomy or D.C.A. is mainly due to a decrease in red cell chlorides (2, 6). The muscle chlorides are only insignificantly increased by D.C.A. or adrenalectomy but show a marked rise in D.C.A.-treated adrenalectomized animals. Progesterone has no such effect. No consistent change has been observed in the chloride concentration of the liver or brain in any of these groups, hence we omitted the corresponding values from our tables. The average circulating blood volume was not significantly changed by D.C.A. in the intact rats of this series because one animal had an unusually low blood volume. However, numerous other experiments lead us to believe that there is a constant increase in the blood volume of normal rats treated with large doses of D.C.A. Thus in a group of four male and four female rats weighing 165 grams on the average, the blood volume was 3.5 ml. while in a similar group of rats treated with 2 mgm. of D.C.A. daily during 47 days, it was 3.9 ml. (P = <0.01). The hematocrit and hemoglobin values showed no significant change, however, so that the condition appears to be one of true plethora. Following adrenalectomy, we observed the usual decrease in blood volume. This could be restored by D.C.A. but not by progesterone.

The blood glucose showed a marked decrease in D.C.A.-treated intact animals while in adrenalectomized rats, D.C.A. tended to restore the low blood sugar towards normal. Progesterone showed no such effect. It should be emphasized, however, that in other experiments D.C.A. failed to cause consistent hypoglycemia. It is noteworthy that progesterone proved entirely inactive in preventing the hypoglycemia of the adrenalectomized animals.

It is evident from the experiments reported above that the same dose of D.C.A. which decreases the blood chlorides in the presence of the adrenals not only fails to do so in the adrenalectomized animal but actually exhibits an inverse action inasmuch as it restores the otherwise low blood chlorides of the adrenal-deficient rat to normal. Tissue chloride determinations failed to throw more light on this curious response since the liver and brain showed no consistent change in chloride concentration under any of the

experimental conditions investigated here. The muscle chlorides revealed only an insignificant rise in D.C.A.-treated intact animals and a much more pronounced rise in adrenalectomized animals in which the blood chlorides were also raised by this compound. Preliminary studies concerning the chloride excretion of D.C.A.-treated rats indicate, however, that concomitantly with the decrease in blood chlorides, the urinary chloride elimination is likewise markedly increased in all cases. This is due partly to a slight and inconsistent rise in the chloride concentration of the urine but mainly to a considerable increase in the total urine output (7).

In order to elucidate the rôle played by the adrenals in influencing the action of D.C.A. on the blood chlorides, another series of experiments was performed on 12 male rats weighing 63 to 85 grams (average 75 grams). Six of these were pretreated with 2.5 mgm. of D.C.A. given twice daily subcutaneously in 0.1 ml. of peanut oil while six controls received only

TABLE 3

Blood chlorides in rats chronically treated with D.C.A.

DAYS	TREATMENT	WHOLE BLOOD CHLORIDES	TREATMENT	WHOLE BLOOD CHLORIDES
0	Oil	413 (392–433)	D.C.A.	422 (398–445)
8		416 (398–456)		353 (346–369)
9	Adrenalectomy		Adrenalectomy	
10	D.C.A.	420 (363–456)	D.C.A.	383 (363–398)
15		395 (374–416)		352 (316–392)
20		333 (316–363)		335 (304–357)

an equivalent amount of peanut oil during the first 8 days of the experiment. On the ninth day, both groups were adrenalectomized. Treatment with D.C.A. was continued in the D.C.A. pretreated group but immediately after the operation the not pretreated controls also received D.C.A. in the same dosage. On the day before adrenalectomy, the blood chlorides in the D.C.A. pretreated group had already decreased considerably below their initial value while those of the not pretreated controls did not change. After adrenalectomy the animals having received D.C.A. only after the operation showed essentially normal blood chloride values on the day following the operation, the D.C.A. having prevented the usual decrease in blood chlorides. In the hypochloremic D.C.A. pretreated group the chlorides actually rose after the operation though not quite to the normal level. These observations are in accord with previously reported findings indicating that adrenalectomy prevents the hypochloremic action of D.C.A.; in fact, they show that the hypochloremia, if already established as a result of D.C.A. pretreatment, tends to disappear

after ablation of these glands. However further continuation of the same treatment indicated that later the blood chlorides decrease to exceedingly low values in both groups even though subsequent autopsy showed no trace of an adrenal remnant. Table 3 summarizes these results.

It is difficult to understand why adrenalectomy temporarily prevents the hypochloremic action of D.C.A. However the known facts are compatible with the assumption that this action of the compound is not a direct one but is mediated by some other organ which in turn is influenced by the adrenals and requires some time after adrenalectomy to recuperate its responsiveness to D.C.A. In view of the fact (7) that after hypophysectomy rats permanently lose their ability to respond to D.C.A. with a decrease in blood chlorides, it is tempting to assume that this action of the hormone is mediated by the hypophysis.

SUMMARY AND CONCLUSIONS

In the intact rat, D.C.A. decreases the whole blood chlorides while in adrenalectomized animals whose blood chlorides are subnormal, the same dose of D.C.A. temporarily raises them to or towards normal. Prolonged D.C.A. administration on the other hand decreases the blood chlorides even after adrenalectomy. The plasma chlorides run roughly parallel with the whole blood chlorides in most cases but their variations are less pronounced than those of the latter. From this it may be concluded that the hypochloremia caused by adrenalectomy or by administration of D.C.A. in normal rats is due mainly to a decrease in red cell chlorides.

As a rule the muscle chlorides are only insignificantly increased by D.C.A. or adrenalectomy but always show a pronounced increase in D.C.A.-treated adrenalectomized animals. The brain and liver chlorides reveal no consistent change after adrenalectomy or after the administration of D.C.A. to intact or adrenalectomized animals.

The blood volume which is decreased by adrenalectomy is restored by D.C.A. administration and in the intact rat D.C.A. causes a slight rise in blood volume.

The blood glucose concentration is decreased by adrenalectomy and restored towards normal, though usually not to normal, by D.C.A.

Progesterone given in doses as high as 10 mgm. per day has no effect on the low blood volume, blood sugar and blood chlorides of adrenalectomized rats in acute experiments. This is of interest because in the case of chronic treatment with this compound other investigators reported progesterone to have essentially the same life-maintaining effect in adrenalectomized rats as D.C.A. has. Since most of the other known physiological actions of these two compounds (progestational proliferation of endometrium, mammary gland stimulation, etc.) are likewise identical, experiments such

as those reported in this communication appear to be especially suitable for pharmacological differentiation between these two compounds. These observations also demonstrate the importance of the —OH group on C-atom 21 for the exertion of the pharmacological actions of these compounds since the substitution of this group by hydrogen is the only chemical difference between the molecules of D.C.A. and progesterone.

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GUM ACACIA AND THE OXYGENATION OF RED CELLS

J. VICTOR MONKE[1]

From the Department of Physiology, School of Medicine, The University of Tennessee, Memphis, and the Department of Physiology, School of Medicine, The University of Maryland, Baltimore

The following five groups of experiments are presented as evidence that gum acacia, in concentrations common to clinical usage, neither inhibits nor retards the rate of oxygenation of red cells in vitro or in vivo. The conclusions drawn from these experiments differ from those of Christie, Phatak and Olney (1), who state that gum acacia, when present in the blood stream, retards the rate of oxygen transport across the plasma membrane of the red cells.

METHODS. The first, fourth and fifth groups of experiments were designed particularly for the present study. The second and third groups were obtained from animals subjected to total plasmapheresis with a gum acacia-red cell-Locke's solution. The data which were obtained from the analysis of the various blood-gum acacia mixtures are recorded in terms of a ratio in which the volumes per cent of oxygen actually found in a test sample is related to the total oxygen capacity of the sample as determined by an analysis of the total hemoglobin. The actual oxygen content of each blood sample was determined by the manometric method of Van Slyke (2). The total hemoglobin content (oxygenated-, reduced-, and met-hemoglobin) was measured as cyanmethemoglobin according to the method of Drabkin and Austin (3). Through the use of proper conversion factors, the value for total hemoglobin so obtained was expressed in terms of its equivalent volumes per cent of oxygen. Blood samples were collected anaerobically by a method described elsewhere (4).

Many of the blood samples may have contained small amounts of met-hemoglobin, since conditions for its slow formation were inherent in some of the procedures. Since the effect being investigated had been stated to be a large one (1), no attempt was made to overcome the smaller sources of error. The oxygen saturation of hemoglobin within the lungs is about 94 to 96 per cent complete. Oxygen saturation values of 90 to 94 per cent in our data may be considered to be within the range normally observed.

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[1] Weaver Research Fellow in Physiology, School of Medicine, The University of Maryland, Baltimore.

OBSERVATIONS. 1. *Red cell-gum acacia mixtures in vitro.* Normal red cells were washed three times in a buffered Locke's solution and, after washing, were deoxygenated in an evacuated flask. Five cubic centimeters of cells were then suspended in an equal volume of each of the various solutions listed in table 1, placed in a rotating tonometer, and oxygenated in a stream of moist air. No attempt was made to obtain the best possible samples of cells since the data were of comparative value only. No evidence was found that the presence of gum acacia in any way influenced the oxygenation of red cells under these conditions (table 1).

2. *Resuspended venous "acacia cells."* In one stage of the experimental work, we obtained small samples of red cells from dogs which had undergone total plasmapheresis. These cells, centrifuged free of plasma, were

TABLE 1

	1	2	3	4	5
	CC. OF CELLS				
	5	5	5	5	5
Suspension solution	5 cc. of serum	2.5 cc. of serum and 2.5 cc. 6 per cent gum acacia in saline	5 cc. of 6 per cent gum acacia in saline	5 cc. of 6 per cent gum acacia in Locke's solution	5 cc. of 6 per cent gum acacia in Ringer's solution
$\dfrac{\text{Oxygen content}}{\text{Oxygen capacity}}$	0.926	0.934	0.936	0.942	0.945

resuspended in a volume of Locke's solution equal to that of the withdrawn plasma, placed in a rotating tonometer, and oxygenated for a period of fifteen minutes in a stream of moist air. The two experiments recorded in table 2 are from a series of six such experiments. In every case, the cells were oxygenated to values well within the normal limits. The low value of the second figure in column 6 in the table is related to an extensive hemolysis in the test sample, and is attributable to the presence of methemoglobin which forms readily when free hemoglobin is present within the blood stream (5).

3. *Oxygen content and oxygen capacity of "gum acacia" venous blood.* Venous blood was withdrawn anaerobically from dogs which had undergone plasmapheresis with a gum acacia-red cell Locke's solution. One portion of the sample was immediately analysed for its oxygen content, while another portion was first saturated with the oxygen of room air in a rotating tonometer and then analyzed. Each value was then related, in

terms of the ratio previously described, to the volumes per cent of oxygen represented by the total amount of hemoglobin present in the sample. The data of table 3 are from one of five such experiments.

The oxygen content of the venous blood obtained just after the operation was very high. This, we believe, was due to the lowered oxygen consumption of the animal during the period of anesthesia under which the

TABLE 2

ANIMAL	TIME	RESUSPENDED "VENOUS CELLS"		
		Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5
P	Before	21.9	21.0	1.042
	After	17.0	19.3	0.883
	2:45	20.6	21.2	0.973
	15:00	20.4	20.8	0.982
	27:30	20.8	20.8	1.000
R	Before	17.3	17.5	0.988
	After	16.0	15.6	1.030
	0:48	13.9	13.2	1.050
	3:15	14.2	14.0	1.010

TABLE 3

ANIMAL	TIME	HEMATO-CRIT READ IN PER CENT	VENOUS BLOOD			OXYGENATED VENOUS BLOOD		
			Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity	Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5	6	7	8	9
W	Before	59	6.91	17.51	0.394	16.74	17.51	0.934
	0:00	56	14.40	19.38	0.744	19.02	19.38	0.947
	1:55		7.09	19.78	0.458	19.90	19.78	1.010
	4:00	55	6.65	19.50	0.431	19.35	19.50	0.990
	6:05	52	6.29	16.00	0.392	17.00	16.00	1.032
	15:30	40	5.76	12.68	0.455	13.00	12.68	1.025

operation was performed (6). Later values were within the normal range. If the gum acacia acts to inhibit the passage of oxygen across the plasma membrane of the red cell (1), one would expect that the volumes per cent of oxygen contained in the venous blood would be higher than normal. This would be a direct result of the statement that the cells did not unload the oxygen due to a restricting surface film of gum acacia. We found no evidence of such restricted movement of oxygen (column 4). As can be

seen in column 7 of table 3, the in vitro oxygenation of the venous blood also resulted in normal values, even though the cells had been in the presence of gum acacia within the blood stream for quite long periods.

4. *Oxygen content of arterial and venous blood in anesthetized dogs.* Normal dogs were anesthetized with dial (60 mgm./kgm.), and branch vessels of both the femoral vein and artery were cannulated in such a manner that the tips of the cannulae extended into the flowing blood stream of primary vessels. Thirty per cent gum acacia in 4.5 per cent saline solution was slowly injected intravenously in an amount such that the gum acacia concentration totalled 1.6 grams per cent of the estimated plasma volume. The deep anesthesia caused by the dial was accompanied with marked respiratory depression which in turn resulted in a rather low degree of oxygen saturation of the arterial blood (column 8, table 4). The data

TABLE 4

ANIMAL	TIME	VENOUS			ARTERIAL		
		Vol. per cent oxygen	Total Hb	$\dfrac{\text{Oxygen content}}{\text{Oxygen capacity}}$	Vol. per cent oxygen	Total Hb	$\dfrac{\text{Oxygen content}}{\text{Oxygen capacity}}$
1	2	3	4	5	6	7	8
	hours						
9-M	Before	8.68	22.50	0.386	20.16	22.50	0.905
Injected 40 cc. of 30 per cent gum acacia in 4.5 per cent NaCl solution to give a calculated plasma concentration of 1.30 per cent							
Dial: 60 mgm. per kgm.	0:15	8.38	21.56	0.389	18.84	21.65	0.872
	2:15	8.06	22.08	0.366	19.56	21.00	0.932
	4:40	9.20	25.80	0.356	21.62	24.92	0.872

shown in table 4 give no evidence that the injection of gum acacia affected the normal relationship between the oxygen content of the venous and that of the arterial blood.

5. *Oxygen content of arterial and venous blood in unanesthetized dogs.* In a final group of five experiments, performed on normal unanesthetized dogs, 30 per cent gum acacia dissolved in 4.5 per cent saline solution was slowly injected intravenously in quantities sufficient to produce a plasma concentration of about 3 grams per cent. Venous blood from the jugular vein and arterial blood from the femoral artery was collected anaerobically at various time intervals after the injection, and was analyzed for its oxygen content and its total hemoglobin. Since this group of experiments is the most decisive in the entire series, two examples are presented in table 5 as evidence that gum acacia produces no demonstrable effect on the oxygen carrying capacity of the red cells.

DISCUSSION. These data indicate that in a variety of conditions, both in vitro and in vivo, gum acacia has no inhibiting or retarding effect upon

the oxygenation of red cells. The small variations, and the occasional larger deviations in the data from a normal level of arterial and venous oxygen values are adequately and satisfactorily explained in terms of the physiological conditions of the moment. Christie, Phatak and Olney (1) have referred only briefly to the clinical signs that led them to suspect deficient gas transport in their patients. It is difficult to evaluate their observations. Studdiford (7), however, describes three instances of severe reactions following the injection of gum acacia into postpartum patients, and describes clinical signs of cyanosis and coma. These, he suggests, may be related to the experimental observations of Christie, Phatak and Olney (1). In view of the findings described in our experiments, we are

TABLE 5

ANIMAL	TIME	VENOUS			ARTERIAL		
		Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity	Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5	6	7	8
	hours						
10-B	Before	7.65	14.60	0.523	13.72	14.31	0.960
Injected 40 cc. of 30 per cent gum acacia in 4.5 per cent NaCl solution to give a calculated plasma concentration of 2.96 per cent							
	0:55	9.10	9.78	0.888	11.54	12.03	0.960
	16:30	7.52	12.42	0.605	13.30	13.62	0.974
	42:00	7.30	14.64	0.512	13.72	14.72	0.932
10-E	Before	14.42	21.60	0.675	18.80	20.15	0.935
Injected 35 cc. of 30 per cent gum acacia in 4.5 per cent NaCl solution to give a calculated plasma concentration of 2.92 per cent							
	0:33	10.54	19.00	0.555	17.00	18.60	0.915
	4:30	12.20	19.30	0.658	17.35	18.15	0.968
	16:50				17.72	18.15	0.952
	43:50				16.85	18.00	0.940

entirely skeptical of the usefulness of data secured after such massive injections of gum acacia as were given by the latter authors. Such injections cause an embarrassing increase in blood volume and probably blood viscosity. We may conclude that the adverse reactions which they describe are most likely explicable in terms of unknown factors peculiar to their study. Amberson (8), in a review of blood substitutes, mentions instances during the first World War, when, in soldiers who had undergone a very severe hemorrhage, gum acacia solutions were ineffectual in sustaining life. He suggested, on the basis of the observations reported by Christie, Phatak and Olney (1), that the injections of acacia into such patients actually may have made their critical condition worse by placing a handicap on their red cells which were already insufficient in number.

At the moment, however, it is safe to conclude that, in each of these instances, the basic cause of death was solely a reduction of the circulating red cells to a level insufficient for the maintenance of life.

Second to whole blood, or lyophilized plasma, gum acacia remains the most satisfactory blood adjuvant available and should be indicated as an emergency measure whenever blood or plasma are not available. Increasing caution and consideration are to be observed, however, when the problem of repeated injections presents itself. The work of Andersch and Gibson (9), and more recently that of Heckel, Erickson, Yuile and Knutti (10), has established the fact that complications beyond control result from multiple injections of gum acacia. A certain amount of gum acacia given in each injection is taken up by the liver and stored as an inert material within the hepatic cells. When repeated injections are given, such large amounts of the gum are eventually stored in the liver that drastic disruption of the normal functioning of the organ is brought about.

SUMMARY

Data have been presented indicating that gum acacia, when injected into the blood stream, does not retard, or in any way affect, the movement of oxygen across the plasma membrane of the red cell. This is in contradiction to observations made and recorded by other authors. Experiments in vitro, as well as experiments on both arterial and venous blood, before and after the addition of acacia, have in no instance shown variations in oxygen saturation of the red cells beyond those found in the controls.

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THE RELATION BETWEEN THE FAT AND CARBOHYDRATE METABOLISM OF LACTATION, AS INDICATED BY THE RESPIRATORY QUOTIENT OF THE MAMMARY GLAND

E. P. REINEKE, W. D. STONECIPHER AND C. W. TURNER

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Although the individual milk precursors withdrawn from the blood stream by the lactating mammary gland have been the subject of numerous investigations, the respiratory quotient of the gland has not received attention until quite recently.

Graham, Houchin, Peterson and Turner (1) reported that the respiratory quotient of the active mammary gland is considerably above unity, suggesting the synthesis of a portion of the milk fat from carbohydrate. Shaw (2) reported that respiratory quotients of the intact mammary gland were difficult to evaluate because of the great variations encountered. However, he stated that the respiratory quotient of the excised perfused gland varies but little from the value of 0.80. This fact together with the claim of Shaw and Petersen (3) that an excess of fat in proportion to other milk precursors is taken up by the active mammary gland was believed to indicate the partial oxidation of fat in the gland. It was postulated that this would account for the lower fatty acids of milk fat.

In view of this rather marked divergence of results, the present experiments were undertaken with the object of establishing more definitely the respiratory quotient of the normal intact gland, as well as some of the factors that will affect this value.

METHODS. Blood samples were drawn simultaneously from the "exteriorized" carotid artery and mammary vein of normal, intact goats. A series of samples obtained without anesthesia and a second series secured following anesthetization of the blood vessels with apothesine were drawn with the animal standing quietly in its stall. However, a certain amount of excitement could not be avoided in about 50 per cent of the cases. Further experiments (unpublished) showed that goats under nembutal anesthesia continue to secrete milk of normal composition at an undiminished rate, and that the uptake of milk precursors by the mammary gland continues at the normal level. Accordingly, large series of samples were drawn from nembutalized animals. The blood samples were collected

under oil into flasks coated with equal amounts of potassium oxalate. They were chilled at once by immersion in an ice water bath, and immediately taken to the laboratory for analysis. Oxygen and carbon dioxide were determined by the manometric method of Van Slyke and Neill (4) and hemoglobin by the method of Evelyn and Malloy (5).

RESULTS. The effect of the various methods of sampling upon the level of oxygen and carbon dioxide in the blood, the respiratory quotient, and the hemoglobin difference is shown in condensed form in table 1. In the

TABLE 1

Showing analyses of arterial and mammary bloods, with calculated respiratory quotients of the lactating mammary gland

NO. OF EXPERI- MENTS	MEAN OXYGEN			MEAN CARBON DIOXIDE			MEAN R.Q.	HEMOGLOBIN PER CENT DIFFERENCE
	Arte- rial	Mam- mary	Differ- ence	Arterial	Mam- mary	Differ- ence		
Series I. Blood samples drawn with no preliminary anesthesia								
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
20	8.49	4.43	+4.07	49.32	53.26	−4.79	1.17 ±0.0361	0.57 (8 values)
Series II. Blood samples drawn with blood vessels anesthetized locally								
15	8.44	4.38	+4.07	50.31	55.03	−4.72	1.15 ±0.034	2.59 (14 values)
Series III. Blood samples drawn under nembutal anesthesia; animals quiet, respiration unimpeded								
29	9.88	5.81	+4.07	53.41	57.84	−4.435	1.09 ±0.0115	1.08 (25 values)
Series IV. Nembutal anesthesia excessive; respiration impeded								
7	9.22	5.16	+4.03	64.06	68.34	−3.21	0.81	3.24 (5 values)

experiments conducted without anesthesia, the respiratory quotient varied between 0.86 and 1.84, with a mean value of 1.17 ± 0.0361. The hemoglobin values obtained in this series showed no significant changes in concentration of the two types of blood. The use of a local anesthetic about the blood vessels (series II) had little effect in reducing the variability of the carbon dioxide:oxygen ratio as shown by the range of 0.92 to 1.68, and mean respiratory quotient of 1.15 ± 0.034. Series III includes the results of 29 experiments in which blood samples were drawn from goats under nembutal anesthesia, with no respiratory complications. When compared to the preceding trials it will be noted that the respiratory quotient is quite constant, varying but little from the mean of 1.09.

It is of interest to note that the average uptake of oxygen by the mammary gland was identical in each of the first 3 series.   Thus any differences in the average respiratory quotient are due to differences in the carbon dioxide exchange.   Furthermore, it seems reasonable to suppose that one of the immediate effects of excitation of the animal would be an increased rate of ventilation of the lungs that would cause abnormal expiration of carbon dioxide.   This in turn would promote a greater transfer of carbon dioxide from the tissues to the venous blood, resulting in a higher carbon dioxide:oxygen ratio.   Anesthetization of the animal before drawing blood samples eliminates this effect and results in much more uniform values.   Further support for this concept is found in series IV, containing the results of experiments in which the anesthesia was excessive, and respiratory difficulties were encountered as a result.   Because of the faulty respiration in these cases there was an abnormally high carbon dioxide level in the blood, which in turn would lead to accumulation of carbon dioxide in the tissues.   Thus the low respiratory quotients recorded are more apparent than real.

The fact having been established that the respiratory quotient of the mammary gland of the lactating goat varies within narrow limits about the mean of 1.09, it seemed of interest to obtain some information on mammary gland metabolism in other physiological states.   Results obtained on non-lactating goats during the last half of pregnancy, in addition to data on one non-lactating-non-pregnant goat and three lactating-fasted goats are presented in table 2.

The rather striking fact comes to light that the respiratory quotient of the mammary gland of the non-lactating-pregnant goat (series V) is identical with that of the goat in normal lactation.   This agrees with the evidence that mammary development is complete and milk secretion begins about mid-pregnancy.

Unfortunately, only one non-lactating-non-pregnant goat was available for sampling, while this investigation was in progress (series VI).   However, all of the ratios fall between the values of 0.68 and 0.98, with the average of 0.81.   It appears, therefore, that the metabolic needs of the inactive gland may be met by the combustion of either fat or carbohydrate, or both, while the active gland normally uses some carbohydrate for fat synthesis.   Another rather interesting point revealed in series V and VI is that the uptake of oxygen by the mammary gland of non-lactating goats is practically the same per unit flow of blood as for normally lactating goats.   Therefore, the increased metabolic requirements of the gland during lactation must be met by a marked increase in the rate of blood flow.

In view of the constancy of the respiratory quotient of the mammary gland during lactation, under the given conditions, it seemed of interest to determine whether or not this ratio would be maintained during fasting, when the carbohydrate stores of the organism would be seriously depleted.

The results of such experiments with three goats are included in series VI. Goats 836 and 444 were in good condition at the start of the fast and their respiratory quotients remained above unity for the first 48 hours,

TABLE 2

Showing analyses of arterial and mammary bloods, with calculated respiratory quotient of nonlactating-pregnant, nonlactating-nonpregnant and lactating fasted goats

GOAT NO.	OXYGEN			CARBON DIOXIDE			R.Q.	HEMO-GLOBIN PER CENT DIFFER-ENCE	DURA-TION OF FAST
	Arterial	Mammary	Differ-ence	Arterial	Mammary	Differ-ence			
Series V. Nonlactating pregnant goats									
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*			*hours*
1	9.41	6.52	+2.89	51.09	54.23	−3.14	1.09	−1.13	
2	10.88	6.91	+3.97	46.35	50.64	−4.29	1.07		
3	10.56	5.97	+4.59	50.25	55.22	−4.97	1.08	+0.64	
3	11.09	5.56	+5.43	46.77	52.64	−5.87	1.08	+1.17	
3	9.12	6.01	+3.11	49.88	53.22	−3.34	1.08	+0.24	
3	9.94	5.56	+4.38	41.66	46.38	−4.72	1.08	−2.66	
3	10.08	6.04	+4.04	46.67	51.02	−4.35	1.08	+0.52	
3	8.82	5.54	+3.28	45.74	49.56	−3.82	1.17	+0.06	
3	9.96	5.32	+4.64	49.42	54.29	−4.87	1.05	+0.67	
Mean ..	9.98	5.94	+4.04	47.53	51.91	−4.37	1.09	0.88	
Series VI. Nonlactating-nonpregnant goat									
4	10.18	5.82	+4.36	54.49	57.21	−2.72	0.62	+0.48	
4	11.31	4.52	+6.79	49.87	56.56	−6.69	0.98	−1.41	
4	10.13	4.35	+5.78	46.45	51.59	−5.14	0.89	−1.21	
4	9.68	5.40	+4.28	53.20	56.39	−3.19	0.75	−0.84	
4	8.38	5.25	+3.13	60.55	63.12	−2.57	0.85	+0.53	
4	10.75	5.32	+5.43	56.17	60.37	−4.20	0.77	−0.27	
Mean ..	10.07	5.11	+4.96	53.45	57.54	−4.08	0.81	0.79	
Series VII. Lactating fasted goats									
5	12.11	8.99	+3.12	51.69	54.93	−3.24	1.04	+0.09	40
6	7.77	6.74	+1.03	56.08	57.30	−1.22	1.18	−3.11	48
7	6.33	4.12	+2.21	52.14	54.05	−1.91	0.87		42
7	9.27	3.69	+5.58	44.68	49.92	−5.24	0.94		66
5	13.67	9.11	+4.56	51.71	55.47	−3.78	0.83		64
6	7.44	5.65	+1.79	57.75	58.97	−1.22	0.68		72
Mean ..	9.43	6.38	+3.05	52.34	55.10	−2.76			

followed by a drop to 0.87 and 0.68, respectively, on the following day. Goat 434 was poorer in body flesh at the start of the fast and her mammary respiratory quotient had declined below unity by the 42nd hour. It

appears likely, therefore, that fat synthesis in the mammary gland continues only as long as ample stores of carbohydrate are available.

DISCUSSION.   In considering the respiratory quotient of the active mammary gland of the goat, presented in table 1, series I, II and III, no support can be found for the claim that fats are partially oxidized and reduced, thus accounting for the lower fatty acids of milk.   On the contrary, the finding of Graham, Houchin, Peterson and Turner (1) that the respiratory quotient of the lactating mammary gland is above unity, indicating the synthesis of a portion of the milk fat from carbohydrate, has been confirmed and extended.   It appears probable that the average value of 1.09 obtained in series III, where the effects of excitement had been eliminated by the use of nembutal anesthesia, very nearly approximates the true oxygen and carbon dioxide exchange in the normal mammary gland. Graham et al. corrected their data for the amount of urea synthesized in the mammary gland, estimating that the average urea output would account for 0.392 vol. per cent of added carbon dioxide.   If we assume that a similar output of urea occurred in the present experiments and add 0.392 vol. per cent of carbon dioxide to the values in table 1, series III, we arrive at a corrected respiratory quotient of 1.18.   In either event the respiratory quotient is sufficiently high, in view of the constancy of the results, to indicate a mammary gland metabolism favoring some fat synthesis from carbohydrate.

It is of interest to correlate the decline in respiratory quotient of the mammary gland during fasting with the changes that are known to take place in the fatty acids of the milk fat during a similar period.   The pioneer investigations of Eckles and Palmer (6, 7) showed that the plane of nutrition has a marked influence on the character of the milk fat.   It was shown that all types of underfeeding cause a decline in the Reichert-Meissl and saponification numbers and an increase in the iodine number, while overfeeding and feeding at the normal plane of nutrition maintained these constants at their normal value.   More recently it was reported by Smith and Dastur (8) that in milk fat produced during a prolonged fast there was a decrease of about 80 per cent in the original content of the lower fatty acids, up to and including C_{14}, a deficiency that was almost entirely made good by an increase in oleic acid.   In view of the parallel decline of the respiratory quotient and the lower fatty acids of milk fat it appears extremely likely that at least a portion of these acids are normally synthesized from carbohydrate.   However, it is also well established that neutral fat derived from the blood stream is available for the needs of lactation.   Thus it appears that the milk fat must be derived from at least two different types of precursors:

1. The higher fatty acids are probably derived from the neutral fat of the blood, as shown by the uptake of neutral fat.

2. The lower fatty acids are formed, at least in part, by synthesis from carbohydrate. The derivation of a portion of these acids from fats of low molecular weight or fat-like fragments that could be taken up from the blood stream in small amounts is not excluded, however.

A further point that may have some significance is the fact that the respiratory quotient of the mammary gland of fasted goats is very similar to the value of 0.80 reported by Shaw (2) and Petersen, Shaw and Visscher (9) for the perfused excised udder. Unless enough is known about the requirements for normal milk secretion to enable the addition of the proper amounts of milk precursor, hormones, etc., the blood passing through the perfusion system would very shortly present a picture of severe inanition insofar as the mammary gland is concerned.

The concept of the synthesis of a portion of the milk fat from carbohydrate implies a further drain on the heavily taxed carbohydrate metabolism of the mammary gland in addition to that needed for lactose synthesis and tissue maintenance. The carbohydrate balances reported by Graham (10) and the ratios of Shaw (2) indicate that the uptake of glucose and lactic acid will account only for the synthesis of lactose. Further experiments from our laboratory (11) indicate that additional carbohydrate, to the extent of about 2.0 mgm. per 100 ml. is taken up by the mammary gland from the blood plasma as a portion of a glycoprotein complex. Since the milk proteins contain only traces of sugar (12), it appears probable that the greater portion of this moiety will be available for use as carbohydrate.

SUMMARY AND CONCLUSIONS

1. The average respiratory quotient of the active mammary gland in 20 experiments on normal unanesthetized goats was 1.17 ± 0.0361. Local anesthetization of the blood vessel sampled in 15 experiments resulted in a mean respiratory quotient of 1.15 ± 0.034. Use of nembutal as a general anesthetic during the sampling of blood greatly reduced the variability of the values, resulting in a mean respiratory quotient of the active mammary gland of 1.09 ± 0.0115. Correction for the synthesis of urea in the mammary gland would increase this figure to approximately 1.18.

2. The average uptake of oxygen by the mammary gland was identical in the three series of experiments noted above. It was suggested, therefore, that the chief cause of variation in the apparent respiratory quotient in unanesthetized animals was due to variations in the rate of ventilation of the lungs, which would cause variations in the amount of carbon dioxide expired.

3. The respiratory quotient of the mammary gland of the non-lactating-pregnant goat during the last half of pregnancy was found to be identical with that of the lactating goat, while that of a non-lactating-non-pregnant goat had declined to 0.81.

4. The mammary gland of goats fasted for periods up to 72 hours and sampled under nembutal anesthesia showed a decline in the respiratory quotient to below unity by the third day.

5. Because of the parallel between the decline of the respiratory quotient of the mammary gland and the known decrease in the lower fatty acids of milk fat during fasting, it is suggested that the synthesis of milk fat from carbohydrate is confined largely to the fatty acids of low molecular weight.

6. The relationship between the synthesis of milk fat from carbohydrate and the carbohydrate balance of the lactating mammary gland is discussed.

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THE RESPONSE OF THE DENERVATED NICTITATING MEMBRANE AND OF BLOOD PRESSURE TO SYMPATHETIC NERVE STIMULATION IN ADRENALECTOMIZED CATS

R. A. CLEGHORN, C. W. J. ARMSTRONG, D. C. AUSTEN AND
G. A. McVICAR

From the Department of Medicine, University of Toronto

In cats moribund from adrenal insufficiency, stimulation of sympathetic vasomotor nerves causes little or no rise in blood pressure. The injection of a variety of pressor drugs, with the exception of epinephrine, is similarly ineffective (Elliott, 1904, 1914; Cleghorn, 1938, 1939; Armstrong, Cleghorn, Fowler and McVicar, 1939). In order to explain this phenomenon, the theory that an adrenal cortical hormone might be concerned in some way with the elaboration of sympathin has been considered. Evidence in favour of such a theory has been advanced by Secker (1937, 1938). He found that the response to repeated stimulation of cervical and mesenteric nerves failed much more rapidly in adrenalectomized cats than in normal animals. In view of this observation, it might be thought that adrenalectomized animals, maintained without cortical hormone, would show impaired responses to sympathetic nerve stimulation. This hypothesis has been tested in adrenalectomized dogs maintained for many weeks without cortin by using a diet high in salt and low in potassium. The rise in blood pressure resulting from splanchnic nerve stimulation in these animals was quite as good as in controls (Cleghorn, Armstrong and Austen, 1938). Further evidence against the position of Secker lies in the finding that cats in severe adrenal insufficiency respond to stimulation of the cervical sympathetic by a strong and sustained contraction of the innervated nictitating membrane (Armstrong et al., 1939). In this report it was suggested that the apparent paralysis of cardioaccelerator and vaso-constrictor nerves in such animals might be due to exhaustion of sympathin at the endings of those nerves. It was felt that sustained hyperactivity of vasomotor pressor mechanisms might result from the diminished blood volume and lead to this state of exhaustion. An account of our efforts to compare quantitatively the liberation of sympathin at vasomotor nerve endings in healthy cats and also in cats in various stages of adrenal insufficiency is presented here.

METHODS. Care of the animals, the method of adrenalectomy, chemical

and other techniques were carried out as described by Armstrong, Cleghorn, Fowler and McVicar (1939). The denervated right n.m.[1] was utilized as the peripheral indicator of sympathin, as described by Rosen-

TABLE 1

Healthy adrenalectomized cats: The effect of epinephrine injections and of sympathin on the contraction of the denervated nictitating membrane

CAT NUMBER	HEIGHT OF RECORDED CONTRACTION OF N.M. IN RESPONSE TO:		
	Epinephrine injections*		Sympathin R. Spl. Stim.†
	0.1γ	1.0γ	
Series A. Cortin-treated			
	mm.	mm.	mm.
1	1	21	15
2	2	25	18
3	11	62	70
4	4	77	88
5	9	44	40
Average	5	46	46
Series B. Acutely adrenalectomized			
6	24		45
7	9		49
8	20		51
9	26	58	69
10	18	31	62
11		33	30
12		30	58
13		38	34
14		43	38
Average	19	39	48

Series A: Cats adrenalectomized 21 to 41 days prior to experiment and maintained by cortin.

Series B: Cats adrenalectomized immediately before experiments.

* Epinephrine injected directly into a femoral vein over a period of 10 seconds in a volume of 1 cc.

† Splanchnic nerve isolated and cut below diaphragm; stimulated supramaximally for 30 seconds; blood pressure rise resulting averaged 44 mm. Hg for series A and 34 mm. Hg for series B.

Contractions of n.m. recorded isotonically; tension 4 grams; magnification ×15.

blueth and Cannon (1932). The n.m. was denervated by excision of the right superior cervical ganglion at least three weeks prior to the experiments in order that maximal sensitivity might be developed (Hampel,

[1] N.m., nictitating membrane.

1934).   It has been found that this sensitivity persists for a minimum of ten weeks.   In the animals in which sympathin liberation was studied some days or weeks after adrenalectomy, the ganglion and right adrenal were removed at one operation.   The second gland was excised a week or two later.   Urethane was used as the anesthetic (Liu and Rosenblueth, 1935) since it permits liberation of sympathin by reflex as well as by direct stimulation of efferent nerves.   In the experiments on animals in adrenal insufficiency, chemical changes in the blood, characteristic of this state, were found.   At autopsy no residual cortical tissue was seen in cortin-treated cats nor in those allowed to develop adrenal insufficiency.

RESULTS.   1. *Examination of Cortin-treated, Adrenalectomized Cats.* (a) *Effect of brief stimulation of sympathetic nerves.*   The n.m. response to splanchnic nerve stimulation appeared to be normal in cortin-treated cats compared to acutely adrenalectomized controls (table 1).   It was also observed that stimulation of a splanchnic nerve for 30 seconds elicited a contraction of the n.m. similar in magnitude to that produced by 1.0γ of epinephrine injected intravenously over a period of 10 seconds.   This quantity of epinephrine produces a submaximal response of the n.m. This finding supplied a means of assaying the amount of sympathin liberated into the blood stream.   The experiments listed in table 1 also demonstrate that the comparison of the sympathin liberated by splanchnic stimulation and the standard 1.0γ dose of epinephrine must be made on the same animal.   The n.m. contractions following splanchnic stimulation in cats 10 and 12 (series B) were exceptionally large compared to the response to epinephrine.   There appeared to be no constant ratio between the effect elicited by 0.1γ and 1.0γ of epinephrine.   Central stimulation of a cut sciatic nerve gave a pressor response about twice that seen with splanchnic stimulation, but the n.m. response was not proportionately greater.   Sympathin liberation in a cortin-treated cat is illustrated in figure 1 a and b.

(b) *Effect of prolonged stimulation of sympathetic nerves.*   Prolonged splanchnic nerve stimulation was carried out in seven cats which had been maintained 13 to 39 days after adrenalectomy by cortin.   The blood pressure remained elevated above prestimulation level for an average of 24 minutes, with a range of 15 to 35 minutes.   In seventeen acutely adrenalectomized controls, the range was 21 to 80 minutes with an average of 41 minutes.   If four controls in which the pressure remained elevated more than 45 minutes were excluded, the average would be reduced to 32 minutes.   The type of anesthetic used—barbiturate or urethane—did not seem to affect the results materially.

It must be recognized that the return of blood pressure to prestimulation level does not signify exhaustion of vasoconstrictor effects.   A marked drop in pressure was observed in several cases on cessation of stimulation

which had been carried on as long as 20 minutes after the return of the blood pressure to prestimulation level. Observations on the denervated n.m. were of little value since contracture occurred in most cases.
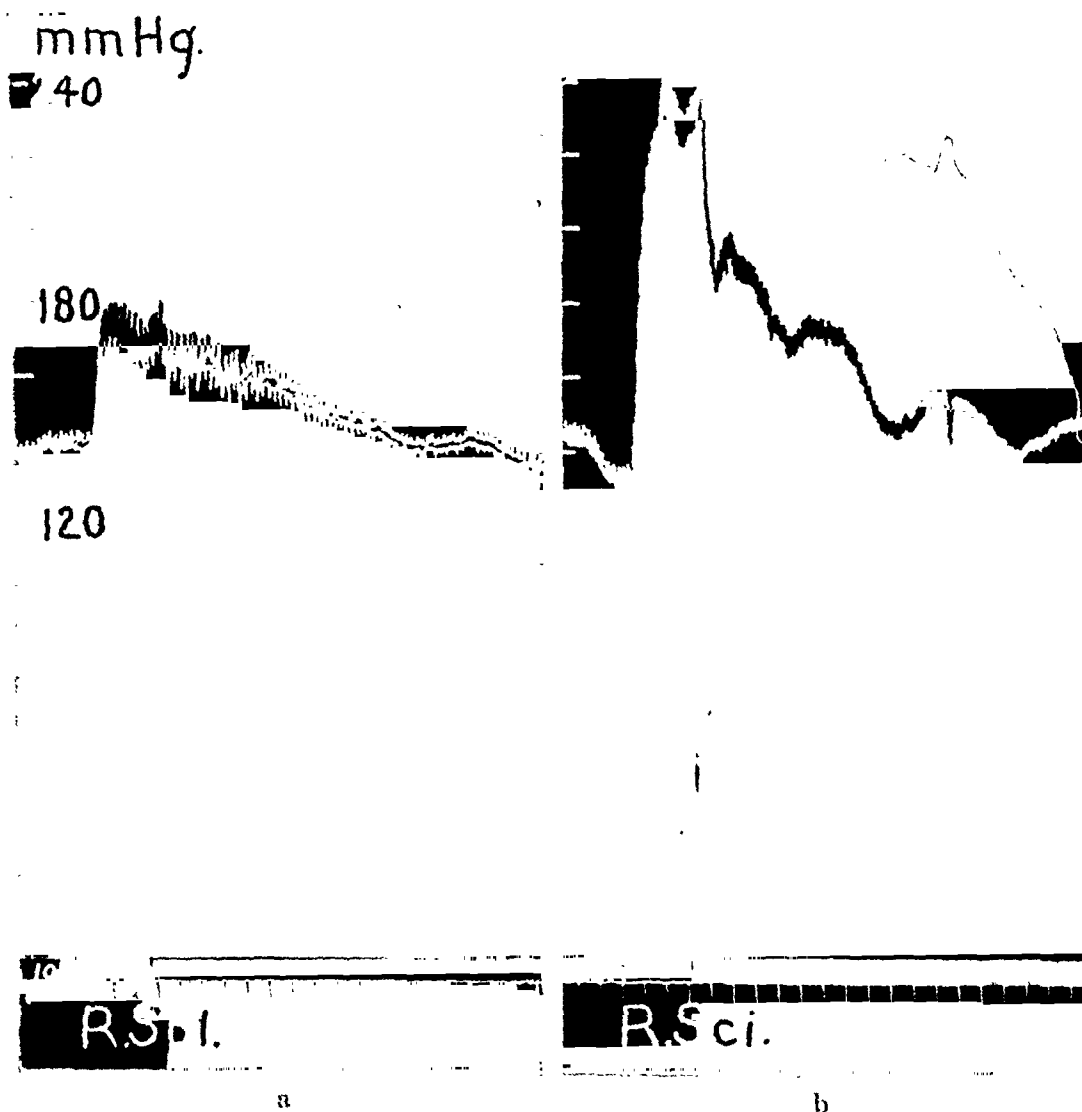
Fig. 1. Nictitating membrane and blood pressure responses in a healthy, adrenalectomized, cortin-treated cat (cat 4, table 1, series A) 25 days after adrenalectomy. Urethane, 1 gram per kgm., intravenously. Upper tracing, carotid blood pressure; lower tracing, denervated n.m.—irregularities due to eye movements.

(a) *R.Spl.*, peripheral cut end of right splanchnic nerve stimulated in abdomen.
(b) *R.Sci.*, central cut end of right sciatic nerve stimulated.

2. *Examination of Cats in Adrenal Insufficiency.* (a) *Effect of brief stimulation of sympathetic nerves.* (i) *Before the development of vascular collapse.* The n.m. response to stimulation of a splanchnic nerve in seven animals examined in either a mild or moderately severe state of adrenal

insufficiency was diminished. The response was reduced absolutely as to magnitude and also relatively with respect to the epinephrine responses. The rise in blood pressure (local sympathin effect), on the other hand, was not significantly less than that found in healthy adrenalectomized, cortin-treated controls. It averaged 41 mm. Hg (table 2, cats 15 to 21). The diminution of the n.m. response in these animals was not directly correlated to the severity of insufficiency nor to the degree of pressor effect obtained

TABLE 2

Cats in adrenal insufficiency: The effect of epinephrine injections and of sympathin on the contraction of the denervated nictitating membrane

CAT NUMBER	DAYS OFF CORTIN	SIGNS OF ADRENAL INSUFFICIENCY AT TIME EXPERIMENT BEGUN				INI-TIAL B.P.	DEGREE OF ADR. INSUFF.	RESPONSE OF N.M. TO: EPINEPHRINE (1.0γ)			RESPONSE OF N.M. TO SYMPATHIN AS PER CENT OF AVERAGE OF RESPONSE TO EPINEPHRINE
		Strength	Weight loss	Rectal temperature	Serum K			Before	Sympathin R. splan.*	After	
			per cent	°C.	mgm. per cent	mm. Hg		mm.	mm.	mm.	
15	10	Not weak	5	36	21.5	170	Mild	65	2	72	
16	9	Weak	6	33	31	130	Mild	23§	0		
17	9	Weak	14	31.5		116	Moderate	52	19	62	33
18	10	Weak	14	33.8	26	112	Moderate	21	14	20	68
19	9	Prostrate‡	12	31.8	27	100	Moderate	26	10	22	42
20	10	Very weak	10	34		80	Moderate	35	6	10	27
21†	10	Weak	13	34.8	28	80	Moderate	16	6	10	45
22	9	Weak	3.3	34.2	29.5	80	Severe	25	0		
23	12	Weak	15	34.4	27	65	Severe	7§	0		
Average..											43

Note: Blank, not done; 0, no response.

* Right splanchnic nerve stimulation for 30 seconds. Blood pressure rise resulting in cats 17–21 (moderate adrenal insufficiency) averaged 42 mm. Hg; in cats 22 and 23 (severe), 0 and 20 mm. Hg respectively.

† See figure 2.

‡ Probably hypoglycemic.

§ To 0.1γ.

by splanchnic stimulation. Five of the cats (nos. 17 to 21) which showed moderately severe adrenal insufficiency are of particular interest. In all these the injection of epinephrine was repeated after the period of splanchnic stimulation in order to control the possibility that the response of the n.m. might deteriorate. In the animals with a blood pressure of 100 mm. Hg or lower (nos. 19, 20 and 21), deterioration in the response of the n.m. to epinephrine occurred. In such animals the average of the contractions

produced by the first and second epinephrine injections gives a more accurate standard with which to compare the n.m. response occurring after splanchnic nerve stimulation. This is expressed as a per cent of the average of the epinephrine responses in the last column of table 2. The average of the assays of sympathin on these animals showed that the sympathin reaching the n.m. was sufficient to cause but 43 per cent of the response shown by the cortin-treated controls (series A, table 1). The pressor response elicited by splanchnic stimulation in these animals averaged a rise of 42 mm. Hg compared to an average of 44 mm. Hg found
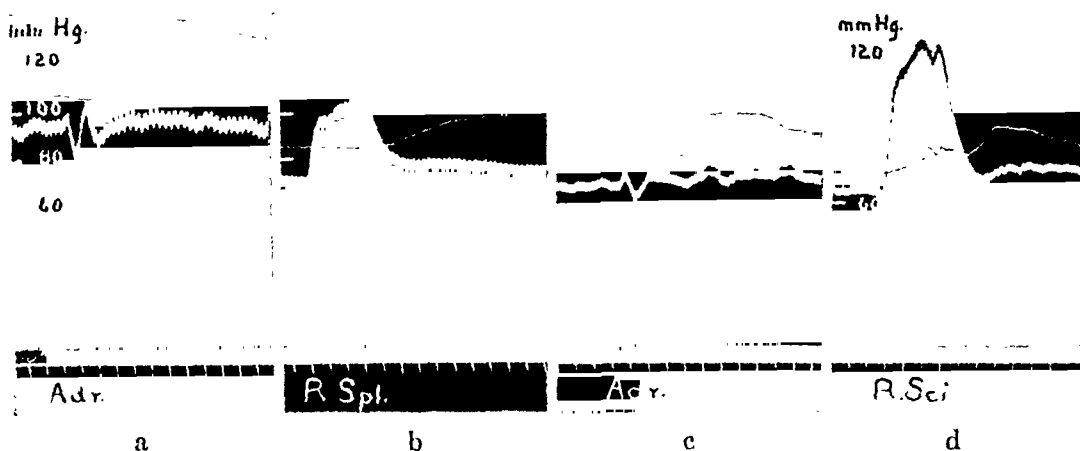
Fig. 2. Nictitating membrane and blood pressure responses in an adrenalectomized cat (cat 21, table 2) in moderately severe adrenal insufficiency 10 days after cessation of cortin injections. Urethane, 0.53 gram per kgm., intravenously. Upper tracing, denervated nictitating membrane (magnification 15); lower tracing, carotid blood pressure.

(a) *Adr.*, epinephrine, 1.0 γ injected intravenously.

(b) *R.Spl.*, peripheral cut end of right splanchnic nerve stimulated in abdomen 21 minutes after epinephrine injected.

(c) *Adr.*, epinephrine, 1.0 γ, injected intravenously 21 minutes after right splanchnic stimulation.

(d) *R.Sci.*, right sciatic nerve (central) cut end stimulated 10 minutes after last injection of epinephrine.

in the cortin-treated controls (series A, table 1). Figure 2, a, b and c, illustrates these points; in figure 2d the response following sciatic nerve stimulation is shown.

(ii) *After the development of severe vascular collapse.* No n.m. response was observed following stimulation of a splanchnic nerve in cats 22 and 23 (table 2). No pressor response to splanchnic stimulation occurred in one, and a rise of but 20 mm. Hg in the other. The blood pressure was then below 30 mm. Hg. A detailed description of the course of the experiment is necessary to understand these results.

Epinephrine was injected shortly after the beginning of the experiment

when the blood pressure was still at the initial level recorded in table 2. This produced a contraction of the n.m. in both cats and also appeared to precipitate a fall in blood pressure since, during the contraction of the n.m., the heart slowed abruptly, the pulse pressure decreased and the blood pressure fell. In cat 22 this fall was from 80 to 26 mm. Hg, and in cat 23 from 65 to 28 mm. Hg. Splanchnic stimulation was done as quickly as possible—within a few minutes of the epinephrine injection—yet no n.m. effect was obtained in either cat; no pressor response in cat 22 and only a 20 mm. rise of Hg in cat 23. No n.m. or blood pressure response occurred on stimulation of a sciatic nerve in either animal.

(b) *Effect of prolonged stimulation of sympathetic nerve.* Prolonged stimulation of the right splanchnic nerve was undertaken in four experi-

TABLE 3

Effect of prolonged splanchnic nerve stimulation on the nictitating membrane and blood pressure in adrenal insufficient cats

CAT NUMBER*	DURATION OF ELEVATION OF B.P. ABOVE PRESTIMULATION LEVEL	NOTE ON B.P. CHANGE	DURATION OF ELEVATION OF N.M. ABOVE PRESTIMULATION LEVEL	NOTE ON N.M. CHANGE
	minutes	*mm. Hg*	*minutes*	
16	15	66–120†	16	Rose after 2½ minutes stimulation. Maximum rise 38 mm.
18	17	61–106†	11	Rose after 1 minute stimulation. Maximum rise 25 mm.
21	15+	67–102‡	9	Rose after 1 minute stimulation. Maximum rise 10 mm.
23	5½	38–64†	0	

* See table 2.
† Fell gradually.
‡ Had fallen to 80 mm. Hg when stimulation terminated at 15 minutes.

ments, in cats 16, 18, 21 and 23 (table 3). The results detailed in this table show that, even in moderately severe states of the vascular failure of adrenal insufficiency, splanchnic stimulation produced sustained effects, as judged by the n.m. and blood pressure responses. Depletion of sympathin at adrenergic nerve endings in the splanchnic region cannot be said to be very pronounced. The n.m. did not show the contracture phenomenon as in the acutely adrenalectomized controls.

DISCUSSION. A normal liberation of sympathin appears to occur in cortin-treated cats for at least 41 days after adrenalectomy. In this series of experiments, brief stimulation of the splanchnic nerve caused comparable effects on the denervated n.m. and on the blood pressure in the cortin-treated group and in the group of acutely adrenalectomized animals used as controls. Prolonged stimulation of the splanchnic nerve caused a

pressor response of slightly shorter duration in the cortin-treated group than in the controls. This cannot, however, be held as evidence for impaired vasoconstriction in response to splanchnic stimulation. Variations in the force of cardiac contraction, in cardiac output or in tone of vessels in regions other than the splanchnic might account for the earlier return of blood pressure to prestimulation level in cortin-treated animals. The n.m. could not be used as an index of sympathin liberation, as contracture occurred. These results confirm the conclusions of Hoskins and Wheelon (1914), Gley and Quinquaud (1918) and others that the excitability of sympathetic vasoconstrictor nerves is not dependent on the presence of the adrenal medulla.

The possible relationship that adrenal cortical extract may have in the formation of a transmitter of sympathetic nerve activity has been discussed at length by Secker (1938). In view of our finding that splanchnic nerve stimulation produces a normal pressor effect in salt-maintained dogs (Cleghorn, Armstrong and Austen, 1938), it seems unlikely that it can have any profound influence on such a function.

In the study of animals in mild adrenal insufficiency (cats 15 and 16, table 2), the sympathin released into the blood stream, as signified by the n.m., was negligible. It is difficult to explain this satisfactorily since, in the cats in moderate insufficiency, contraction of the denervated n.m. was observed on splanchnic stimulation. However, the amount of sympathin liberated in these experiments (cats 17 to 21) appeared to be much less than that shown by the controls, as judged by the effect on the n.m. Since the pressor responses obtained in these animals were normal, compared to the cortin-treated controls, it seems that the amount of sympathin released and effective locally was not diminished. Two separate bits of evidence indicate that the amount of sympathin released after splanchnic stimulation in these cats was not diminished: first, the standard 1γ dose of epinephrine also gave a contraction of the n.m. which was consistently smaller than in the controls; second, we have found, in unreported experiments, that 1γ of epinephrine injected over a 30 second period gives a n.m. contraction of only about half that elicited by the same amount injected over a 10 second period. It is obvious that the concentration reaching the n.m. is important. We believe that the diminished n.m. response in these animals may be explained by the diminished circulation rate characteristic of this stage. This would result in greater dilution of the sympathomimetic substance and also result in a longer period available for the destruction of the liberated sympathin or the injected epinephrine (Bain, Gaunt and Suffolk, 1935; Gaddum, 1938). The advent of this stage is not associated with any change in blood chemistry but with a fall in blood pressure below a level of about 115 mm. Hg. Confirmation of the hypothesis that diminished circulation rate leads to the reduced n.m.

responses is contained in figure 2. Progressive impairment of the circulation rate was observed during the course of the experiment. The n.m. response to the second dose of epinephrine was smaller and showed a latent period following the injection, twice as long in figure 2c as in figure 2a.

Probably the portal circulation is slowed even more seriously than the systemic in such animals. As emphasized by Armstrong et al. (1939), the gastrointestinal viscera show intense congestion in adrenal insufficiency. This fact makes it seem likely that the time taken for sympathin released in the splanchnic region to reach the n.m. must be considerably extended and the opportunity for dilution increased. The amount of sympathin released under these circumstances, therefore, may not be reduced so much as would appear from our findings.

Cats in the stage of severel adrenal insufficiency, when the blood pressure has fallen to shock levels, show little or no pressor response to splanchnic nerve stimulation. This phenomenon has been discussed by Armstrong et al., 1939. They considered failure of ganglionic function as a cause and dismissed it. Measurement of intestinal volume has shown that vasoconstriction does occur on stimulation of a splanchnic nerve in severe adrenal insufficiency despite the absence of a rise in blood pressure (Cleghorn, Fowler and Wenzel, 1939). The blood pressure fails to rise, therefore, either because the heart cannot raise the pressure against the increased peripheral resistance or because of the flaccid state of the peripheral vascular system in regions other than that supplied by the stimulated splanchnic nerve. We believe that the former is probably the more important factor because there is evidence of cardiac malfunction in both cats and dogs dying of adrenal insufficiency, even when the animal is undisturbed. In cats, a terminal decrease in heart rate has been observed (Cleghorn, 1938). This coincides with the terminal fall in pressure. In dogs, cardiac irregularities and bradycardia are frequent late characteristics (Hall and Cleghorn, 1938).

SUMMARY

In healthy, adrenalectomized cats, maintained by cortin for 21 to 41 days, the n.m. and blood pressure responses to brief excitation of sympathetic nerves were found to be as great as in controls acutely adrenalectomized. Cats deprived of cortin for 9 or 10 days and showing signs of mild or moderately severe adrenal insufficiency showed an impaired n.m. response to brief splanchnic nerve stimulation but a normal pressor response. The standard dose of epinephrine (1γ) produced a smaller n.m. contraction in animals in moderate insufficiency than in controls. No n.m. contraction and little pressor response to splanchnic stimulation occurred when the blood pressure had fallen to shock levels. It is concluded that exhaustion of sympathin, at least in the splanchnic region, is not the cause of the vascular failure in adrenal insufficiency.

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THE EFFECT OF RATTLESNAKE VENOM (CROTALIN) ON THE PLASMA HISTAMINE OF THE RABBIT

A. L. CHUTE AND E. T. WATERS

From the Department of Physiology, University of Toronto

The similarities of anaphylactic shock and histamine shock and the release of histamine during anaphylactic shock convinced some investigators that this liberation of histamine was fundamental and directly responsible for the most dramatic of the responses which follow the injection of the shock dose of antigen into the sensitized animal. The similarities of anaphylactic shock and histamine shock are especially well marked with certain species, the dog, guinea pig and rabbit. With the first two species anaphylactic shock is accompanied by a very significant increase in the histamine concentration of the blood. But, unexpectedly, the rabbit responds differently, for these animals in anaphylactic shock show no increase, but rather a decrease in blood histamine (Rose and Weil, 1939; Dragstedt et al., 1940). This decrease in histamine of the whole blood may be attributed to the characteristic leucopenia of anaphylaxis, for as Code (1937) has shown, the blood histamine resides for the most part in the leucocytes. Now the rabbit contains relatively large amounts of histamine also in its plasma. Rose and Weil report a decrease in 2 experiments and a small increase in 2 experiments in the plasma histamine of the rabbit in anaphylactic shock.

Histamine studies have also been made on animals shocked with snake venoms, including rattlesnake venom. It has been shown that the addition of rattlesnake venom to the fluid perfusing isolated guinea pig lungs leads to a release of histamine from these organs (Feldberg and Kellaway, 1937) and intravenous injection of venom into the dog results in an increase in blood histamine (Dragstedt and Mead, 1938), though in smaller amounts than in comparable conditions of hypotension in anaphylactic shock. It therefore appeared to us to be of interest to determine what the effect of rattlesnake venom (Crotalin) would be on the plasma histamine of the rabbit; whether there is an increase as in certain species, or a decrease as in acute anaphylactic shock of the rabbit.

METHODS. The rabbits were fasted for 24 hours. Blood samples (8–9 cc.) were taken by cardiac puncture. A small drop of heparin solution was placed in the syringe to prevent coagulation. The blood was im-

mediately centrifuged and 3 cc. of plasma pipetted into 7.5 cc. 10 per cent CCl_3COOH. After standing for about an hour the precipitate was removed by filtration and well washed. It was found, when the mixture was left in the refrigerator for a day or more and then filtered, that there was a considerable loss of histamine. The histamine was assayed by the method of Code (1938).

Dried rattlesnake venom (Crotalus horridus)[1] was dissolved in a 50 per cent aqueous solution of glycerol, such that 1 cc. contained 3 mgm. of the dried venom. The injections of $\frac{1}{3}$ cc. of this solution were made slowly ($\frac{3}{4}$ to 1 min.) into the ear vein of the rabbits. The results are tabulated.

TABLE 1

RABBIT NO.	WEIGHT	INJECTION	HISTAMINE		OBJEC-TIVE SIGNS
			Before	After	
	kgm.		$\gamma/cc.$	$\gamma/cc.$	
1	2.9	First	0.33	0.18 (12 min.)	—
2	2.85	First	0.77	0.29 (14 min.)	++
3	2.65	First	0.15	0.0 (21 min.)	
4	2.3	First	1.05	0.30 (5 min.)	++
	3.0	Second, 2 months later	0.41	0.07 (14 min.)	++
		Third, 1 day later	0.25	0.12 (17 min.)	++
5	2.5	First	0.23	0.15 (20 min.)	+
		Second, 2 days later	0.22	0.20 (25 min.)	++
6	2.0	First	0.50	0.40 (9 min.)	+
				0.30 (60 min.)	
		Second, 2 months later	0.58	0.16 (18 min.)	—

All our estimations have been made on plasma. It will be seen that in all experiments there is a definite decrease in the amount of histamine present in the plasma a short time after the first intravenous injection of the venom. A second injection of venom, some time later, into one rabbit produced no change in the plasma histamine. Two other rabbits receiving subsequent injections showed the same substantial decrease in the concentration of histamine as after the first injection. In most of the experiments the animals voided urine and feces, sometimes with diarrhea, a few minutes after the injection of venom; one animal, however, became incoördinate in its movements for a few minutes. Apart from this one exception the animals showed only mild signs of distress. An attempt

[1] We are indebted to our colleague, Dr. J. Markowitz, for the venom.

was made to make a rough evaluation of these symptoms, as will be seen from the table. The plasma of the blood samples taken subsequent to the injection of venom was frequently slightly hemolysed and cloudy in appearance.

SUMMARY

The intravenous injection of rattlesnake venom into rabbits produces a decrease in the concentration of histamine in the plasma of these animals. It is apparent that the rabbit possesses a mechanism for the relatively speedy removal of histamine from the blood, quite apart from leucopenia. We suggest that in Crotalin shock, and probably anaphylactic shock and related phenomena, histamine may be released as in animals of other species, but because of the efficiency of this removal mechanism (in the blood or other tissues) of the rabbit, even the normally circulating histamine largely disappears. .

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THE CIRCULATION IN REST AND WORK ON MOUNT EVANS (4,300 M.)

ERLING ASMUSSEN AND FRANK C. CONSOLAZIO

From the Fatigue Laboratory, Harvard University, Boston

In a previous paper Asmussen and Chiodi (1940) demonstrated anew that the cardiac output of man in rest and during work is increased when the O_2-content of the inspired air is lowered, and evidence was brought forward to show that it was not the oxygen-lack *per se*, but the lowered arterial O_2-tension, which through the chemoreceptors of the carotid glomus stimulated the circulatory center. Emphasis was put on the possibility that the response of the body to *prolonged* (i.e., lasting more than a few hours) O_2-deficiency might differ from that described, and the works of Grollman (1930) and Christensen and Forbes (1937) were mentioned as examples of how prolonged exposure to low barometric pressure causes changes in the circulation rate, which cannot be explained by the stimulating effect of the low arterial O_2-tension alone. These authors found that although individual differences were apparent in their subjects, the general feature was an increased cardiac output in the first few days of an exposure to low O_2-tension, followed later by a decrease, which brought back the cardiac output practically to sea-level values.

Grollman correlated this return with the concomitant increase in O_2-capacity of the blood. However, an inspection of his data and curves shows that the greatest increase in O_2-capacity occurred during the first three days, as had been observed by Schneider (1921), whereas the decline of the cardiac output began after five or six days. In order to find an explanation of the different levels of the cardiac output in the first days and in the later stages of a sojourn at high altitude, we have studied both the percentage and total quantity of hemoglobin in the blood, the blood volume and the composition of the alveolar air. The cardiac output was determined not only in rest but also during work of known intensity. It seemed likely that such data might add useful facts to our knowledge of circulatory regulation.

LOCALITIES AND SUBJECTS. The University of Denver has for several years had a laboratory building at the top of Mt. Evans, Colorado (4,300 m.), which we were enabled to use through the kind coöperation of Dr. J. C. Stearns, Professor of Physics at the University of Denver.

The barometric pressure there during the first two weeks of August, 1940, was about 460 mm. Outdoor temperatures ranged from a minimum of 0° in the early morning to a maximum of about 20° in the afternoon. We arrived at Mt. Evans August 3 in the late afternoon and started our experiments next morning. One of us (F. C., aged 27; weight, 86 kgm.; height, 172 cm.) came directly from Boston by train. E. A. (aged 33; weight, 68 kgm.; height, 171 cm.) had spent four days in Denver (elevation 1,700 m.) and one night in Idaho Springs (elevation 2,300 m.), and furthermore had paid a short visit to Mt. Evans on August 2. Both subjects were in good physical condition upon arrival, but experienced mountain sickness during the first 48 hours. Sleeplessness, vomiting, diarrhea, precordial pains, palpitation, etc., at night were followed by a slight headache during the day. The rest of the time both were in good health, except during the last three days when E. A. began to develop a gastro-intestinal ailment, which however did not become pronounced until after leaving the mountain.

PROCEDURES AND METHODS. The general scheme for the day was: Experiments on both subjects (fasting) from about 8 a.m. to 1 p.m. Lunch and rest to about 2:30 p.m. Analysis and other laboratory work to about 6 p.m. An outdoor walk or climb to about 7 p.m. Supper and rest to 8 p.m. Calculating, dishwashing, reading, etc., to about 10 p.m. All determinations thus were made in the fasting state.

Metabolism in rest and during work was determined from the ventilation, measured on an accurate dry gas meter, and from the composition of the expired air, drawn from a mixing chamber between the valve and the gas meter. Alveolar air samples were taken in rest by the Haldane-Priestley method, during work as samples from the expiration chamber of the valve at the end of each expiration. All samples were analyzed on the Krogh-Haldane apparatus.

Cardiac outputs were estimated from the metabolic rate and the arteriovenous O_2-difference, determined by the Grollman method. As in previous experiments (Asmussen and Chiodi, 1940), we have chosen to use only those determinations in which the average O_2-tension during the rebreathing comes within a few millimeters Hg of the previously determined alveolar O_2-tension. Pulse rates were counted on the wrist and blood pressures (taken only in rest) measured in the ordinary way on the left arm.

For the determination of oxygen capacities blood was drawn from a cubital vein, saturated with O_2 in a tonometer at room temperature and analyzed in the Van Slyke manometric apparatus. The blood volume was estimated by the CO method: A measured volume (usually 170 cc. at 760 mm. Hg, 0°, dryness) of CO was mixed with O_2 and a little N_2 in a small rubber bag and rebreathed for 20 minutes, the CO_2 being absorbed in soda-lime. A sample of blood was then drawn from the cubital vein and

analyzed for CO after Van Slyke and Neill (1924). Used in this way the CO-method gives good checks with the dye method (Evans' blue) as ordinarily used in this laboratory. Its advantage is that it is very simple and can be used every day, as the elimination of the CO in our experience seemed to be complete in 24 hours, whereas the dye persists in the plasma for 10 to 12 days. From the arterial O_2-capacity and the blood volume the total O_2-capacity could be calculated.
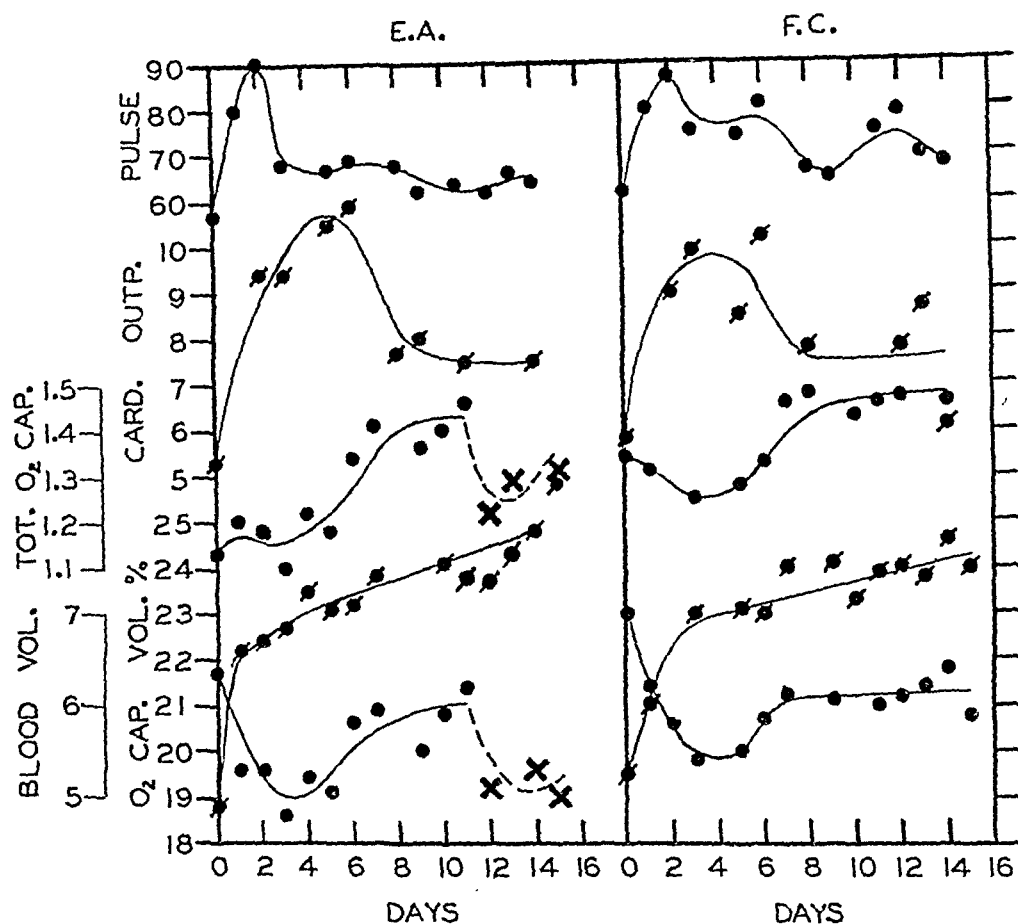
Fig. 1. Pulse rate, cardiac output (barred dots), total oxygen capacity, oxygen capacity in volumes per cent (barred dots), and blood volume for E. A. and F. C. during 15 days at 4,300 m. Crosses represent abnormal values of blood volume and total oxygen capacity (see text).

For the working experiments a simplified Krogh bicycle ergometer was used (Christensen and Forbes, 1937). Two grades of work were investigated, a lighter work (330 mkg./min.) and a heavier work (490 mkg./min.). A metronome secured a constant rhythm of 60 revolutions per minute.

RESULTS. The results of the resting experiments on E. A. and F. C. are shown in figure 1. The dots represent the actual determinations, whereas the curves are drawn so as to smooth out the daily irregularities. In

E. A. the blood volume—and consequently the total O_2-capacity—on the 12th day for an unknown reason dropped down to values almost as low as in the first days. These values, which might have been caused by a developing ailment, as mentioned above, are shown by crosses instead of dots. We did not get any values of the cardiac output on the first day because of difficulties in finding the right acetylene-oxygen mixture. For the following days a marked initial increase in the cardiac output and a later secondary fall to values still significantly higher than the sea-level values was found in both subjects. It will be noticed that the O_2-capacity in volumes per cent increases very rapidly the two first days, later slowly

TABLE 1

DAYS AT 4300 M.	E. A.			F. C.		
	Alveolar		A.-v. O_2-difference	Alveolar		A.-v. O_2-difference
	pCO$_2$	pO$_2$		pCO$_2$	pO$_2$	
	mm. Hg	mm. Hg	cc./l.	mm. Hg	mm. Hg	cc./l.
(Sea-level)	42	96	47	43	101	50
1	29.1	44.3		33.1	37.9	
2	28.2	45.0	30	30.8	43.7	33
3	30.6	44.3	30	31.6	46.1	30
4						
5	29.0	50.3	27	33.4	43.9	35
6	29.8	47.3	26	29.7		29
7						
8			37			38
9	28.5	49.9	35	29.4	49.4	41
10						
11	28.1	49.8	38			
12				27.6	49.6	38
13						34
14			38			45

but steadily, whereas the *total* O_2-capacity seems to be rather stationary, or even falling a little, the first 5 or 6 days, after which it starts increasing with the above-mentioned exception for the last few days in E. A. The blood volume was markedly below sea-level values on the first day and continued to decrease for two days more. While it then began to increase it did not attain, even in F. C., who remained in good health, the sea-level value. While the pulse rate, as shown in figure 1, shows considerable changes in the first days, the blood pressure was constant and showed no significant differences from the sea-level values. Table 1 contains some additional data. It will be noticed that the alveolar pO$_2$ increases slightly and the alveolar pCO$_2$ decreases slightly during the sojourn at Mt. Evans,

indicating that the resting ventilation was increasing slightly during the process of acclimatization.

The resting metabolism was constant from day to day but, especially in E. A., somewhat increased as compared with the sea-level values. This may depend on the rather low temperatures in the morning, when the determinations were made, and therefore is more pronounced in E. A., who is rather lean, than in F. C. The possibility that the increased ultra-violet radiation, as mentioned by Lindhard (1910), might be another reason for this cannot, however, be neglected. The average values were for E. A., 283 cc./min., for F. C., 297 cc./min., compared with respectively 250 and 289 cc./min. at sea-level.
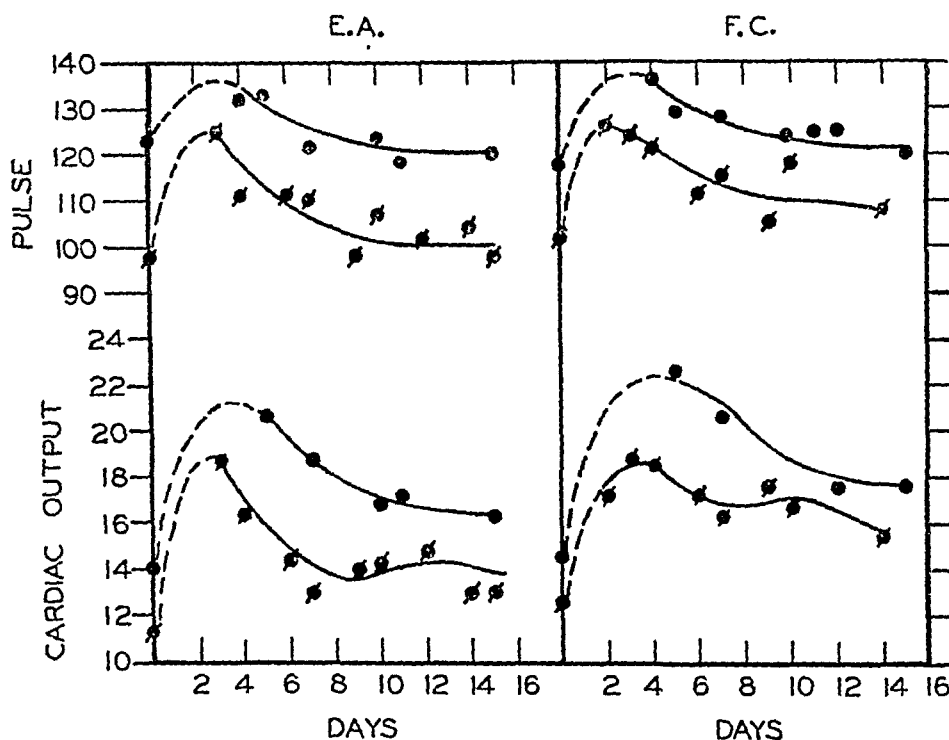
Fig. 2. Pulse rates and cardiac outputs for E. A. and F. C. in two grades of work during 15 days at 4,300 m. Dots: 490 mkg./min. Barred dots: 330 mkg./min.

The pulse rates and cardiac outputs for the two grades of work are shown in figure 2. The corresponding average metabolisms are, for E. A. 1.11 l./min. in the lighter work, 1.44 l./min. in the heavier work, for F. C. resp. 1.23 l./min. and 1.61 l./min. The efficiency of E. A. thus was higher than that of F. C. These values correspond closely to the sea-level values for both subjects. The ventilation in the steady state of work increased in both subjects during the process of acclimatization, and corresponding changes in the composition of the alveolar air were observed.

DISCUSSION. The results of our resting experiments show qualitatively great resemblance to those of Grollman (1930). Quantitatively, however,

our results differ from Grollman's. Whereas the maximal increase in Grollman's two subjects was less than 50 per cent and the final value reached was the normal sea-level value, both of us showed an initial increase of about 100 per cent, and the final values reached were still significantly higher than our sea-level values. The only possible explanation seems to be that the discrepancies must be due to individual differences.

The decline in cardiac output after a certain time spent in high altitude has been correlated by Grollman with the well-known increase in O_2-capacity of the blood which occurs during a sojourn in high altitude. Grollman's figure 1 and our figure 1, however, show that although the greatest increase in O_2-capacity takes place in the first 1 to 2 days, the cardiac output nevertheless continues to rise thereafter, and the decline starts at a time when the increase in O_2-capacity is relatively slow and uniform.

The variations in the cardiac output correspond, however, in both our subjects rather closely to the observed changes in blood volume, as figure 1 shows.

An interdependence between blood volume and circulation rate has been asserted before. Thus Scott, Bazett and Mackie (1940) have demonstrated that during the first few days of a sojourn in a hot room the cardiac output is slightly increased, but that it assumed normal values again when the blood volume later was increased. The interdependence of the blood volume and the cardiac output is apparently a rather slow adjustment: When blood pools in the lower extremities, as in passive standing, an increased cardiac output is not evoked, but frequently the opposite (Asmussen, Christensen and Nielsen, 1939, 1940). However, for longer exposures—several hours or days—the problem must be different: the compensatory vasoconstrictions must build up a "want for oxygen," which no doubt will be met by an increased circulation rate. The very high cardiac output found in the first week thus might be explained as partly due to the low blood volume during the same time, and partly to the low O_2-tension. When the blood volume and the total Hb of the blood again increase, a decrease of the cardiac output can be tolerated and a new level is obtained.

The question now is whether the decline in blood volume (plasma volume) should be looked upon as a failure of the organism to maintain a status quo, or whether it rather should be looked upon as a regulatory device. For the former point of view speaks the fact that both of us were mountain-sick the first night and day and probably lost some water through vomiting and diarrhea. But then again it should have been easy to regain this loss of water during the following days, when no ailment was present. As this did not happen we are inclined to believe that the second suggestion is the more likely and to assume that the decline in plasma volume is a

regulatory mechanism by which the O_2-capacity of the blood is increased. This interpretation of the event has been given first by Abderhalden (1896), later by Douglas, Haldane, Henderson and Schneider (1913). The subsequent increase in blood volume is, as a simple calculation will show, for a great part, due to the increased number of erythrocytes. The significance of a high O_2-capacity of the blood for maintenance of a normal capillary O_2-tension has been discussed by Barcroft (1925) and Haldane and Priestley (1935) and needs no further explanation.

The level reached after these initial stages is in our case higher than the sea-level values. It appeared to us to be significant to determine whether in this state the usual controller of the cardiac output in low O_2—i.e., the arterial O_2-tension—was still active. We therefore in the last two days at Mt. Evans, in a few experiments on both subjects (1 during light work and 2 in rest), added O_2 to the inspired air to such a degree

TABLE 2

STATE OF SUBJECT	E. A.				F. C.			
	A.-v. O_2-difference		Cardiac output		A.-v. O_2-difference		Cardiac output	
	Rest	Work	Rest	Work	Rest	Work	Rest	Work
Acclimatized at 4300 m. (alveolar pO_2 = 50 mm. Hg)....	38	88	7.4	12.9	39	80	7.6	15.5
Acclimatized at 4300 m. (alveolar pO_2 = 150–200 mm. Hg)	60	116	4.7	9.8	64	127	4.6	9.8
Sea-level (alveolar pO_2 = 100 mm. Hg)	47	99	5.3	11.5	50	97	5.9	12.8

that the alveolar pO_2 increased from the normal, about 50 mm. Hg, to 150–200 mm. Hg. After 20 to 30 minutes' O_2-breathing, the cardiac output was determined. Table 2 shows the results.

The decrease in the cardiac output when the alveolar pO_2 was increased shows that the chemoreceptors must have been stimulated by the prevailing low O_2-tension also in the acclimatized subjects.

Still another observation can be made from the data of table 2: The cardiac output during the O_2-breathing was lower than in the same subjects at sea-level (compare Grollman (1930) after descent to Manitou). A supernormal alveolar O_2-tension in subjects at sea-level has no effect on the circulation rate (Grollman, 1932, confirmed by experiments in this laboratory). The difference between a subject acclimatized to high altitude and one acclimatized to sea-level, both placed at sea-level, is therefore comparable to the difference between a normal subject and a patient with anemia, both at sea-level O_2-pressure. H. E. Nielsen (1934)

has followed the recovery of a patient with pernicious anemia through two months, and found that the cardiac output gradually decreased to normal values as the O_2-capacity gradually increased. Christensen (1937), from H. E. Nielsen's results, has calculated the average venous O_2-tension, which—according to many others (e.g., Barcroft, 1925; Haldane and Priestley, 1935)—approximates the average O_2-pressure in the tissues, and points out that this is kept practically constant with Hb percentages ranging from 36 per cent to 82 per cent. Grollman (1932) likewise points out that in his experiments the venous O_2-tension in low O_2 is approximately normal, and the results of Asmussen and Chiodi (1940) confirm this. The maintenance of a constant venous O_2-tension, indicating a constant tissue O_2-tension, seems therefore to be a very important function of the circulatory adjustments.[1]

The results of our experiments in work (fig. 2) need no long discussion. The general trend seems to be the same as in rest, so that the working experiments can be said to confirm the conclusions drawn from the resting experiments.

Our results emphasize the complexity of the regulation of the cardiac output. Besides the well-known rôle of the chemoreceptors and the pressoreceptors, the capillary pO_2, the O_2-capacity, the blood volume, etc., are important. Even though their effect may be slower and therefore less striking than that of a lowered arterial pO_2 it may be as important. A more complete study of their interrelation with the circulation may give valuable information concerning the regulation of the circulation rate.

SUMMARY

Two subjects were studied in rest and work during a sojourn of 15 days at the summit of Mt. Evans, Colorado (elev. 4,300 m.). In both subjects an increase in the resting cardiac output of about 100 per cent occurred during the first 4 to 5 days, later followed by a return to values still well above sea-level values.

The blood volume decreased considerably the first 3 or 4 days, then increased to a level still below the sea-level values. The percentage O_2-capacity was increased already the first day, presumably as a result of withdrawal of fluid from the blood. Determinations of the total O_2-capacity showed that an increased formation of red cells first occurred after a latency of 4 to 5 days.

In work the cardiac output was considerably higher the first days than

[1] The experiments of Asmussen and Chiodi (1940) show that *acute* (about 1 hour) exposures to low tissue O_2-tensions, as in CO-poisoning, do not increase the cardiac output. Whether a prolonged CO-poisoning would do so still has to be proved, but seems likely considering the increased cardiac output of patients with pernicious anemia.

later on, but even then it was still higher than at sea-level. The decline in cardiac output after about a week at high altitude is presumably related to the concomitant increase in blood volume and total O_2-capacity.

The chemosensible reflexes were active during the whole sojourn at Mt. Evans. If the acclimatized subjects breathed an air mixture enriched with O_2, the cardiac output both in rest and during work fell to values lower than the corresponding sea-level values.

The findings are discussed and brought into relation with other results on the regulation of the circulation.

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THE REVERSIBILITY OF pH EFFECTS ON THE O₂ CONSUMPTION OF TISSUES[1]

A. WARREN STEARNS, Jr., MILTON GREENBLATT, ATTILIO CANZANELLI
AND DAVID RAPPORT[2]

From Tufts College Medical School, Boston Mass.

In an earlier communication (1939) we have recorded observations of the effect of changes of pH on the in-vitro O_2 consumption of certain isolated tissues. Briefly, from this work it appeared that the oxygen uptake of guinea-pig liver, kidney and testis was constant over a wide range of pH, far beyond the limits of viability of the organism as a whole. In brain the curve showed less of a plateau, and there was indeed a rather sharp maximum of oxygen consumption at about pH 9.0 to 9.5. Beyond the ranges of pH at which the oxygen uptake of the tissues was normal or above normal, the Q_{O_2} fell more or less sharply to zero, at approximately pH 4 on the acid side and between pH 10 and 11 on the alkaline side. The implications of these results, particularly in respect of the relation of aerobic oxidations to viability, made it of interest to investigate the effects of changes in pH further. The present communication deals with the reversibility of the pH effects in different tissues.

METHODS. The methods employed were similar to those of the paper referred to above. The medium was in all cases horse serum, made almost bicarbonate free by shaking with HCl at about pH 6.0 to 6.5, and adding either HCl or NaOH to bring to the desired pH. The pH of the medium was determined by means of a glass electrode before the tissues were added to the respiratory flasks, at the end of one hour of tissue respiration, and at the end of the experiment. In some experiments both the control tissues which had been respiring at physiological pH and those at test pH were transferred to other flasks containing the medium at physiological pH. In the majority of experiments a further control was carried out, namely, that at the end of an hour about one-half of the tissue at the test pH was transferred to other flasks containing medium at physiological pH, as above, the remainder continuing to respire at the test pH. These

manoeuvres permitted us 1, to compare the initial respiration at control (physiological) pH and at the various test pH's; 2, to determine the relative change in Q_{O_2} as a function of time at the various pH levels; 3, to determine whether a tissue which had been transferred from a medium at a test pH to one at physiological pH had a higher O_2 consumption than it had originally had at the test pH (this we have designated "absolute recovery") or whether it had a higher O_2 consumption than it would have

TABLE 1

Guinea-pig kidney

CONTROL Q_{O_2} AT CA pH 7.3		TEST pH	TEST Q_{O_2}			REVERSIBILITY	
			1st hr.	2nd hr.		(c)/(a) "Absolute" recovery	(c)/(b) "Relative" recovery
1st hr.	2nd hr.		(a) At test pH	(b) Remaining in medium at test pH	(c) Transferred to medium at ca pH 7.3		
						per cent	*per cent*
17.5 ±0.1	16.6 ±0.1	4.3	6.2 ±0.6	5.6 ±0.3	7.3	118	130
22.3 ±0.6	20.2 ±0.1	4.5	4.7 ±0.4		4.0 ±1.0	85	
15.0 ±0.1	15.4 ±0.2	4.7	9.1 ±0.1	6.4 ±0.1	10.4 ±0.4	114	163
19.1 ±0.8	17.7 ±0.0	4.9	9.8 ±0.5		13.0 ±0.3	133	
17.3 ±1.7	16.6 ±1.0	5.2	13.7 ±1.3		15.5	113	
15.8 ±0.1	15.1 ±0.2	5.2	11.4 ±0.4	10.0 ±0.0	12.6	111	126
19.7 ±0.8	16.5 ±0.6	5.6	15.1 ±1.2		16.9 ±0.7	112	
15.4 ±0.8	14.4 ±0.2	5.8	13.0 ±0.4	11.9 ±0.4	14.4 ±0.8	111	121
20.7 ±1.7	19.5 ±0.1	6.1	16.6 ±0.8		20.2 ±0.9	122	
18.9 ±0.8	17.6 ±1.0	6.1	15.4 ±1.0		20.6 ±1.5	134	
19.4 ±1.2	18.5 ±0.4	6.8	18.8 ±1.0		19.9 ±1.2	106	
18.7 ±1.1	16.7 ±0.7	8.6	18.2 ±0.3		16.8 ±0.2	92	
15.8 ±0.1	15.1 ±0.2	8.8	15.3 ±0.3	14.3 ±0.6	13.0 ±0.1	85	91
15.4 ±0.8	14.4 ±0.2	9.3	14.9 ±0.0	13.9 ±0.3	12.1 ±0.4	81	87
18.3 ±1.0	16.4 ±0.3	9.3	12.8 ±0.5		12.6 ±0.3	98	
15.0 ±0.1	15.4 ±0.2	9.7	13.6 ±0.3	11.6 ±0.4	11.6 ±0.3	85	100
16.7 ±0.2	15.8 ±1.0	10.0	4.8 ±0.1	2.8 ±0.3	3.4 ±0.3	71	121
19.6 ±0.5	18.1 ±1.0	10.1	3.4 ±0.5		0.4 ±0.1	12	
17.5 ±0.1	16.6 ±0.1	10.2	7.5 ±0.1	2.4 ±0.7	5.0 ±0.4	67	208
16.7 ±0.2	15.8 ±1.0	10.3	3.8 ±0.5	1.7 ±0.1	2.0 ±1.0	53	117

had if it remained at the test pH (this we have designated "relative recovery").

As in the experiments previously reported a correction was made at pH's above 9.5 for the oxygen consumption of the serum medium itself, though this is not great until about pH 10.5 is reached.

RESULTS. 1. *Kidney*. The experiments on this tissue are summarized in table 1. On the acid side of neutrality, experiments were carried out over a range extending to pH 4.3. At all pH's tested below 6.8, the Q_{O_2} at the test pH was lower than the normal at physiological pH, and became

progressively less as the acidity of the medium was increased. The effect of time on this phenomenon was negligible, as the diminution of the O_2 uptake during the second hour was no greater than in the normal controls. However, that part of the tissue which was removed at the end of the first hour from the acid medium, and put into a medium at physiological pH, showed unmistakable recovery. It will be seen in table 1, in the column headed "relative recovery," that the Q_{O_2} of this transferred tissue was greater than that of the same tissue remaining at the test pH, as will be seen from the fact that the ratio (c)/(b) is always greater than 100 per cent. Not only, however, was there an arrest of the deterioration process when the tissue was transferred to the medium at physiological pH, but there was definite evidence of a reversal of effect, for as will be seen from the column headed "absolute recovery," the O_2 uptake of the transferred tissue was constantly greater than the original O_2 uptake at the test pH, as indicated by the fact that the percentile ratio (c)/(A) was consistently greater than 100. Indeed, as will be observed in the table, the respiration when the tissue was returned to the normal medium was in several cases higher than that of the same tissue that had remained in the normal medium throughout the experiment. We are therefore confronted with a phenomenon which in this case is definitely reversible.

At alkaline pH's this is not true. As we have previously shown, there is no appreciable effect, initial or otherwise, until the neighborhood of pH 9 is reached. Beyond that point, such interference with aerobic oxidation as is brought about by changes in the pH of the medium seem to be permanent, in the sense that they are not reversed when a tissue is transferred to a medium at normal pH. There is, however, some evidence of "relative" recovery, that is, of arrest of the deterioration, though this arrest was at best incomplete.

2. *Liver.* While not so striking as in the case of the kidney, there was, in the experiments in guinea pig liver, again evidence that the effect of increasing the hydrogen ion concentration of the medium on the tissue respiration is a reversible one. Only at pH 4.6—the lowest tested—was the O_2 uptake of the tissue transferred from the acid medium to the one at physiological pH less than that of the tissue at the test pH during the previous experimental period, and on the average it was eight per cent higher (table 2). The change was small, and might have been questionable had it not been reasonably consistent. The "relative" recovery over the Q_{O_2} of the tissue remaining at test pH was in all cases large and unmistakable.

At alkaline pH's the evidence indicated clearly, as in the case of kidney, that the effect of decreasing the hydrogen ion concentration (which occurred at pH's above 10) was permanent. There was no sign of "absolute" recovery, and for the most part there was not even much evidence that a

return to a normal medium had any tendency to arrest the deterioration of oxidation.

3. *Brain.* This tissue, unlike both kidney and liver, showed no indications of a reversibility of the effect on oxygen uptake of lowering the pH, as will be seen in table 3. Only an arrest of the deteriorating effect on

TABLE 2

Guinea-pig liver

CONTROL Q_{O_2} AT CA pH 7.3		TEST pH	TEST Q_{O_2}			REVERSIBILITY	
			1st hr.	2nd hr.			
1st hr.	2nd hr.		(a) At test pH	(b) Remaining in medium at test pH	(c) Transferred to medium at ca pH 7.3	(c)/(a) "Absolute" recovery	(c)/(b) "Relative" recovery
						per cent	*per cent*
6.4 ±0.1	6.6 ±0.2	4.6	3.0 ±0.3	1.3 ±0.5	2.6 ±0.3	87	200
7.6 ±0.2	8.4 ±0.2	5.2	4.8 ±0.1	3.3 ±0.2	6.0 ±0.3	125	182
9.3 ±0.1	7.4 ±0.1	5.2	4.5 ±0.5	2.5 ±1.0	4.6 ±0.1	102	184
7.9 ±0.9	6.6 ±0.6	5.4	4.2 ±0.1		4.5 ±0.3	107	
6.8 ±0.1	8.0 ±0.6	5.6	4.7	3.3	4.7 ±0.2	100	143
8.7 ±0.3		5.6	5.6 ±0.2	4.3 ±0.3	6.2 ±0.4	111	144
5.7 ±0.2	6.4 ±0.1	5.9	4.7 ±0.3		5.2 ±0.1	111	
6.1 ±0.1		6.1	4.8 ±0.2		4.9	102	
8.4	8.1 ±0.5	6.1	5.5 ±0.2		6.3 ±0.2	115	
8.5 ±0.1	10.2 ±0.1	6.3	5.9 ±0.1	4.8 ±0.3	6.1 ±0.5	103	127
9.0 ±0.1	8.2 ±0.3	6.4	6.5 ±0.4	4.4 ±0.1	7.4 ±0.2	114	168
5.7 ±0.2	6.4 ±0.1	7.9	6.5 ±0.2	6.1 ±0.4	6.1 ±0.5	94	100
8.7 ±0.3		8.5	9.4 ±0.4	9.4 ±0.4	8.8 ±0.3	94	94
9.3 ±0.4	9.1 ±0.8	9.3	10.2 ±0.7	9.3 ±0.8	8.1 ±0.4	79	87
6.1 ±0.1		9.4	6.4 ±0.2		4.4 ±0.0	69	
7.6 ±0.2	8.4 ±0.2	9.7	8.2 ±0.2	7.2 ±0.5	6.7 ±0.0	82	93
8.4 ±0.1	8.9 ±0.2	10.0	8.6 ±0.3	6.9 ±0.1	6.9 ±0.1	80	100
8.4 ±0.1	8.9 ±0.2	10.2	6.7 ±0.1	4.4 ±0.3	4.9 ±0.5	73	111
7.6 ±0.2	8.1 ±0.4	10.3	8.7 ±0.3	5.2 ±0.7	5.7 ±1.0	65	110
8.5 ±0.1	10.2 ±0.1	10.5	8.6 ±0.3	3.2 ±0.2	4.0 ±0.1	47	125
8.7 ±0.4	8.8 ±0.5	10.5	5.5 ±0.1	3.1 ±0.8	3.4 ±0.3	56	110
6.8 ±0.1	8.0 ±0.6	10.6	4.1 ±0.2	0.8 ±0.4	2.7 ±0.2	66	+
7.6 ±0.2	8.1 ±0.4	10.6	6.6 ±0.3	3.4 ±0.7	4.9 ±0.3	74	144
6.4 ±0.1	.6.6 ±0.2	10.9	1.7 ±0.6	0.0	1.3 ±0.4	76	+
8.7	8.8	10.9	1.9 ±0.7	0.0	1.1 ±0.2		

returning the tissue to a normal medium, as shown in the last column of this table, is to be observed.

On the alkaline side, our previously reported experiments had shown a precipitous fall in the O_2 consumption from a level above normal to zero at about pH 10.5. In those experiments we were fortunate in being able

to record several intermediate points in this fall. In the present research this proved more difficult, but we can report two experiments in which there was no "absolute" recovery from about a 50 to 60 per cent depression of metabolism, as well as four others in which a fall to even lower levels proved to be irreversible. At the highest pH levels there was some slight evidence of arrest of the deterioration when the tissue was returned to a medium at normal pH.

TABLE 3

Guinea-pig brain

CONTROL Q_{O_2} AT CA pH 7.3		TEST pH	TEST Q_{O_2}			REVERSIBILITY	
			1st hr.	2nd hr.			
1st hr.	2nd hr.		(a) At test pH	(b) Remaining in medium at test pH	(c) Transferred to medium at ca pH 7.3	(c)/(a) "Absolute" recovery	(c)/(b) "Relative" recovery
						per cent	*per cent*
8.4 ±0.4	6.8 ±0.1	4.4	1.8 ±0		1.0 ±0	56	
9.1 ±0.1	7.5 ±0.3	5.0	2.7 ±0.1	1.0 ±0.1	1.5 ±0.1	56	150
8.0 ±0.3	6.1 ±0.1	5.6	7.1 ±0.1	3.8 ±0.2	4.6 ±0.2	65	121
6.5 ±0.3	5.6 ±0.1	5.9	5.9 ±0.5	4.0 ±0.5	5.5 ±0.2	93	138
7.2 ±0.0	5.5 ±0.1	6.3	6.2 ±0.0	5.0 ±0.1	6.2 ±0.1	100	124
8.6 ±0.0	7.0 ±0.3	8.3	8.5 ±0.1	7.8 ±0.4	7.8 ±0.0	92	100
8.6 ±0.0	7.0 ±0.3	8.6	8.0 ±0.1	7.0 ±0.2	7.0 ±0.2	88	100
6.5 ±0.3	5.6 ±0.1	9.1	7.2 ±0.2	6.7 ±0.1	6.1 ±0.5	85	91
9.1 ±0.1	7.5 ±0.3	9.4	9.5 ±1.1	8.0 ±0.6	7.3 ±0.7	77	91
8.0 ±0.3	6.1 ±0.1	9.6	9.2 ±0.5	6.9 ±0.2	5.6 ±0.3	61	81
7.2 ±0.0	5.5 ±0.1	9.7	6.8 ±0.1	4.9 ±0.1	4.3 ±0.3	63	88
8.6 ±0.0	7.0 ±0.3	10.1	3.9 ±0.6	0.9 ±0.2	3.2 ±0.7	82	+
7.6 ±0.1	5.9 ±0.1	10.1	1.1 ±0	0.3	0.3		
7.6 ±0.1	5.9 ±0.1	10.2	1.0 ±0.0	0.6	0.2		
7.0 ±0.2	6.1 ±0.4	10.3	1.9 ±0.5	0.6 ±0.3	1.0 ±0.3		
8.4 ±0.4	6.8 ±0.1	10.5	3.1 ±0.2	0.4 ±0.4	1.9 ±0.2	62	+
7.0 ±0.2	6.1 ±0.4	10.5	1.0 ±0.3	0	0.9 ±0.1		
8.4 ±0.4	6.8 ±0.1	10.9	0	0.4 ±0.4	0.8 ±0.3		

As in the case of the other tissues, there was no indication that a change in pH, even if beyond the limits of viability of the organism, would have an effect on the O_2 consumption during the second hour of an experiment, if it had not already had such an effect during the first hour. This confirms observations made in our previous paper on this subject, and tends to support the hypothesis expressed in that paper that failure of the vital functions of the organism resulting from a change in pH is not caused by interference with either anaerobic or aerobic oxidations.

SUMMARY

Observations on the reversibility of previously described effects of changes in pH on the respiration of tissues in-vitro have demonstrated the following:

. On the acid side of neutrality, the diminution of oxygen consumption which occurs at pH levels beyond the viability limits of the organism is reversible in the case of guinea-pig kidney and liver even when the pH is as low as 5. In the case of kidney the recovery may be complete. This reversibility is not seen in the case of the brain. Even in the latter, there is however some tendency towards arrest of the deterioration in respiration.

On the alkaline side of neutrality, the diminution of respiration as a result of changes in pH, beginning at about pH 9.5 to 10, are irreversible in all the tissues studied, though the effects may be to some extent arrested by returning the tissues to a medium at normal pH.

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CANZANELLI, A., M. GREENBLATT, G. A. ROGERS AND D. RAPPORT. This Journal 127:·290, 1939.

ERRATUM

Volume 132, page 10, line 32, KCl, 0.650 per cent should read KCl, 0.0450 per cent.

THE MECHANISM OF VAGAL EFFECTS ON PULMONARY VENTILATION

T. E. BOYD

From the Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago

Since expiration involves relatively little muscular activity, and may be entirely passive (Gesell, 1936), the work of breathing is mainly inspiratory. Gradual filling of the lungs, in a normal inspiration, is accomplished by a muscular tetanus of progressively increasing strength. Consequently the rate of energy expenditure must increase throughout the act, and successive equal increments are added to inspiratory volume at increasing cost. Any increase of inspiratory depth, velocity remaining constant, entails extra work which is out of proportion to the added tidal volume (Rohrer, 1925). It is often assumed that the minute volume of air inspired measures the rate of activity of the respiratory center; but if this is true the activity of the center does not always conform to that of the respiratory muscles. Any given minute volume can be maintained by varying combinations of depth and frequency. A reduced frequency, however, cannot be offset by a reciprocal adjustment of depth without increasing the total energy expended.

Gad (1880) suggested that the motor discharge to the inspiratory muscles is proportional to the mean inspiratory volume rather than to ventilation rate. On a graph relating lung volume to time, the mean inspiratory volume in a given cycle is measured by the area included between the graph and the base line of expiration, divided by the duration of the cycle. Over a one-minute period the total discharge would be proportional to the area of an average cycle multiplied by frequency (referred to below as the "minute area"). This area in apneusis, as an extreme example, would obviously reflect the rate of inspiratory activity, and the stimulating effect of CO_2, somewhat better than the ventilation rate. For normal breathing, the validity of Gad's index appears to be confirmed in the recent studies

of Gesell, Magee and Bricker (1940) on the temporal distribution of inspiratory action potentials. They find, throughout inspiration, a steady recruitment of new motor units into activity, with some increase in the frequency of firing of those already active. The rate of discharge (number of unit potentials recorded per unit of time) rises along a gradient which corresponds to the rate of filling of the lungs. The mean rate of discharge, over any given period of time t, must then be approximately proportional to the mean volume over the same period; and the total discharge (mean rate $\times t$) proportional to mean volume $\times t$. The latter product is the area referred to above.

After vagal section the minute volume may fall (Hammouda and Wilson, 1932), particularly if the animal is already in a state of hyperpnea from rebreathing (Rice, 1938). Central stimulation of one vagus, so timed that it cuts short each inspiration near the normal level, reverses this effect. Respiratory frequency (Hillenbrand and Boyd, 1936) and minute volume (Hammouda and Wilson, 1939) are augmented. The latter authors infer that these changes of minute volume must result from parallel changes in the motor discharge; hence, that intermittent vagal stimulation actively excites the center and that vagal section removes a tonic excitatory influence. Since the altered ventilation is accompanied in each case by reciprocal changes of rate and depth, it seems to us, for the reasons outlined above, that the minute area might give more reliable information concerning the actual variations of inspiratory activity. We have therefore made comparative measurements of minute area and minute volume, from graphic records of our own and from those presented as typical by Hammouda and Wilson (1932, p. 86).

PROCEDURE. Dogs were used, a combination of barbital-sodium (0.25 gram per kgm.) with pentothal sodium (15 mgm. per kgm.) being given intravenously. In 8 experiments intrapleural pressure was recorded instead of lung volume. Over a wide range inspiratory volume varies in direct ratio to negative pressure around the dog's lung (Cloetta, 1913), and the latter can be recorded photographically, avoiding the inertia to which nearly all forms of volume recording apparatus are subject in some degree. Through the chest wall was introduced one arm of an L-shaped glass tube, of 3 mm. bore. This part of the tube had several openings, and was adjusted to lie in the intrapleural space parallel to the ribs. The outside arm of the tube was connected to a segment capsule with a rubber membrane. In 4 experiments the dog (weighing 9 to 11.6 kgm.) was placed in an oblong box of about 98 liters capacity. The trachea was connected through its cannula to the outside air and the box sealed. The small pressure changes in the closed air space of the box, caused by and proportional to the respiratory changes of lung volume, were recorded optically.

The film records were enlarged to about 25 times their original area, and traced on paper. The base line of expiration was made continuous. The paper used (Keuffel and Esser graph, 358-14) was found to be of sufficiently uniform quality to permit comparative area measurements by the method of cutting out and weighing. Area measurements made by weight, on 20 squares of 25 sq. cm. each cut from separate sheets, gave a probable error of 0.17 sq. cm. On each record the depth of several consecutive inspirations was measured, and the average depth multiplied by frequency. The area was measured on the same graphs, and the average

TABLE 1

Comparison of minute volume and minute area, both expressed in arbitrary units
(see text)

I, data from Hammouda and Wilson, 1932, figure 2 and accompanying table. II, data from figure 1 of this paper.

	FRE-QUENCY, PER MIN.	$N \times$ DEPTH, OR MINUTE VOLUME	MINUTE AREA
I			
	(n)		
A. Normal, before vagal section......................	12	100	100
B. Immediately after section........................	5	159	238
C. 30 minutes later..................................	4.5	73	119
D. Later, effect of rebreathing.......................	8	349	482
II			
A. Rebreathing, left vagus intact....................	32	100	100
After section of left vagus........................	17	64	193
B. Before vagal stimulation........................	6	100	100
During vagal stimulation.........................	15	89	29
C. Before vagal stimulation........................	5.5	100	100
During vagal stimulation.........................	10	123	71
D. Before vagal stimulation........................	7	100	100
During vagal stimulation.........................	13.3	110	46

multiplied by frequency to give minute area. These data give minute volume and minute area in arbitrary units, the initial or control value being taken as 100 for comparison in the tables.

RESULTS. *a. The effect of vagotomy.* Section of both vagi, if the animal is breathing quietly at the time from outside air without abnormally large dead space, is usually followed by an immediate increase of gross minute volume (confirming Rice, 1938, and Hammouda and Wilson, 1932, 1939). Later the minute volume is gradually reduced, reaching a new steady level. The minute area is also immediately augmented after vagotomy, relatively more than minute volume. Later the area also falls, but it always re-

mains, even after the new steady state is reached, above the pre-vagotomy figure. We have found no exceptions to this. It may be illustrated by our measurements on the graphs of Hammouda and Wilson (1932, p. 86), presented for comparison with their data on minute volume (table 1 of this paper). The total inspiratory discharge per minute evidently is augmented after vagotomy, even after the breathing has become stabilized. Minute volume fails to increase in proportion, because the deep and prolonged inspiration is relatively inefficient. The augmented inspiratory activity can be attributed simply to the loss of vagal inhibition. In quiet breathing the ventilation is initially increased, in spite of lowered efficiency. The intensity of chemical stimulation is thereby lowered, and this leads in turn to a reduction of inspiratory activity until a new equilibrium is reached.

If, on the other hand, the animal is already hyperpneic from rebreathing at the time of section, the minute volume falls immediately (confirming Rice, 1938), but the minute area nevertheless increases sharply (fig. 1, A, and table 1). The fall of ventilation is therefore not due to a reduced motor discharge, but to relatively ineffective grouping of an augmented discharge, as in apneusis.

b. *The effects of intermittent central stimulation of the vagus.* Brief tetanic stimuli, beginning at a fixed stage in each inspiration, were applied to the central stump of one vagus after section of both. The circuit was automatically controlled by the animal's breathing, in the manner described by Hillenbrand and Boyd (1936). The stimuli were of a strength just sufficient to cut short inspiration.

By this procedure, the frequency of breathing is regularly augmented (fig. 1, B, C, D). There is, however, a limit to the degree of acceleration thus attainable. When inspiration is cut short at a very early stage, acceleration is not enough to offset the lowered tidal volume, and minute volume is reduced (fig. 1, B, and table 1). If inspiration is allowed to reach a more advanced stage, the acceleration is somewhat less but minute volume is increased (fig. 1, C and D, and table 1). In either case, however, the minute area is reduced. The increase of minute volume results from more effective grouping of a diminished inspiratory discharge. The effect is similar, in this respect, to that of intermittent central stimulation of the vagus in apneusis (Pitts, Magoun and Ranson, 1939a).

In 3 of the series of 12 experiments stimulation of the vagus during inspiration occasionally failed to inhibit. Instead, there was an increase in velocity and depth of the current inspiration. One or two inspirations of a group were affected in this way, the others being cut short as usual. This inspiratory response was noted by Gesell and Moyer (1935). It is not abolished by local cooling of the nerve to 8°C., whereas the inhibition of inspiration, and the accelerator effect of intermittent stimulation, are

both lost (Hammouda and Wilson, 1939). Presumably, therefore, the irregular inspiratory reflex is mediated by a distinct fiber group, not concerned in the rate and ventilation effects here under consideration.
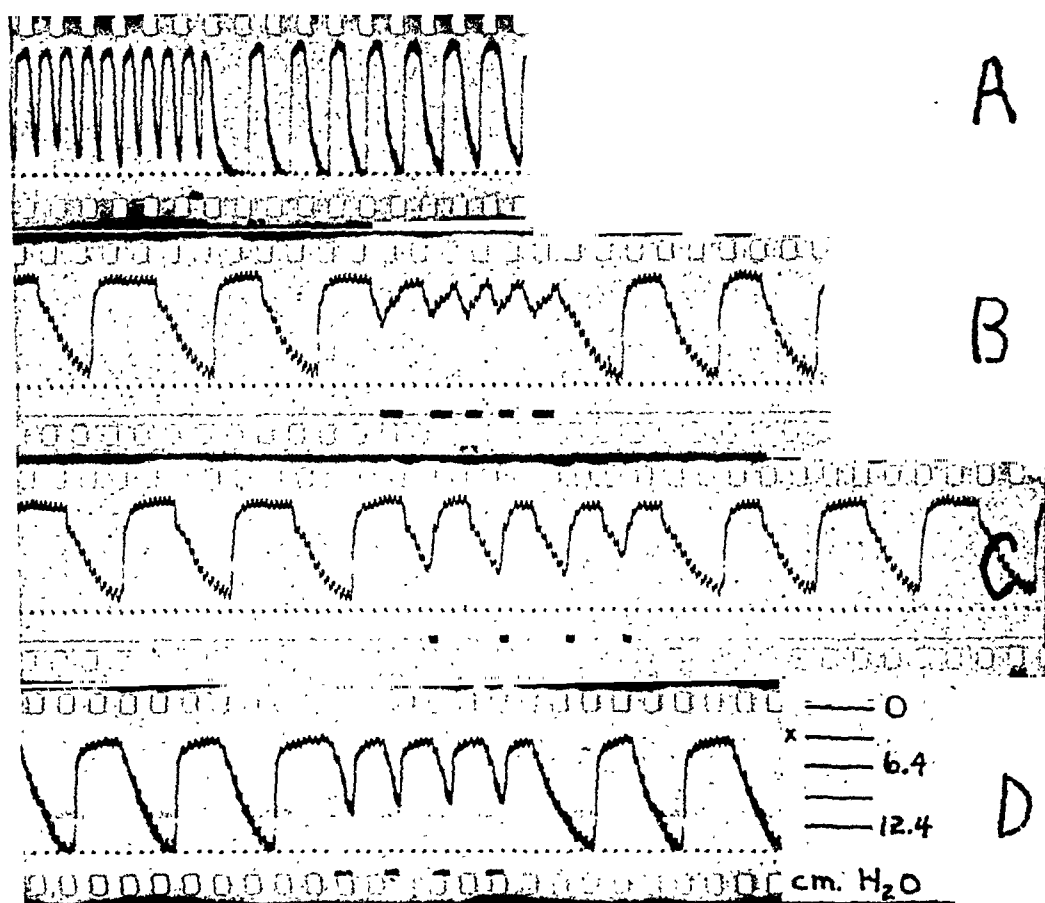
Fig. 1. Records of intrapleural pressure. Time in seconds. Calculation of data given in table 1.

A. Dog, 14.8 kgm., breathing through tube of 100 cc. capacity attached to tracheal cannula. Right vagus sectioned previously. At signal, section of left vagus. The first cycle following is obviously distorted by mechanical stimulation from section, and is not included in data tabulated.

B. Dog, 12.5 kgm. Both vagi sectioned. Signals, central stimulation of left vagus.

C. Same, stimulation applied at a later stage of inspiration.

D. Same animal as in A, later, after removal of extra dead space and stabilization of breathing. Signals, central stimulation of right vagus.

DISCUSSION. With respect to vagal section, the present work merely confirms the contention of Gad (1880), that the act of inspiration becomes wastefully prolonged; and that the total activity of the respiratory motoneurones is invariably increased, without a corresponding increase of ventilation rate. This effect of vagotomy appears in hyperpnea as well

as in quiet breathing. The waste of energy may be exaggerated if inspiration is prolonged in time out of proportion to depth (as in fig. 1 A of this paper), but it occurs even if depth and duration are augmented in the same proportion. Normally, according to the calculations of Rohrer (1925) rate and depth are reciprocally adjusted in such a way that adequate ventilation is secured with a minimum expenditure of energy. This requires the finding of an optimum between rapid, shallow breathing, which is ineffective because of the dead space, and deep slow breathing, which is uneconomical.

The condition brought on by simple vagotomy appears to differ from apneusis only in degree. In the former condition the waste of inspiratory energy is limited, because inspiration is still cut short, though at a relatively advanced stage, by the pneumotaxic center (Stella, 1938a, 1938b; Pitts, Magoun and Ranson, 1939a, 1939b). When the pneumotaxic center also is eliminated, inspiration is further prolonged and still less efficient. In either condition, periodic central stimulation of a sectioned vagus, so timed as to imitate and replace the lost inflation reflex, can augment frequency and minute volume while reducing the total inspiratory discharge per minute.

The net influence of the vagi on the central mechanism is thus inhibitory, in the sense that it keeps the motor discharge tonically restrained. Gesell (1940a, 1940b) believes that the vagal inflation reflex accelerates the velocity of inspiration. He considers it to be purely excitatory, a positive drive exerted at first on the inspiratory motoneurones and abruptly transferred, at a certain stage of inspiration, to the expiratory side. When this shift takes place, inspiration is cut short by reciprocal inhibition. Admitting that such a mechanism is possible, the fact remains that the expiratory muscles contribute relatively little to the total energy expended in breathing, and the vagal expiratory drive can therefore add but little to the total motor discharge. In terms of the latter, the expiratory drive is far outweighed by the reciprocal inhibition of inspiration. And granting that the rate of inspiratory recruitment may be slower after vagotomy, the total inspiratory discharge, in each cycle and per minute, is nevertheless increased.

SUMMARY

1. The motor discharge to the inspiratory muscles, over a given period of time, is measured approximately by the mean inspiratory volume (Gad's index), or the area included between the base line of expiration and the graph relating lung volume to time.

2. Section of the vagi results in augmented inspiratory activity. This occurs no matter whether the animal is breathing quietly, or is hyperpneic from rebreathing, at the time of section. The impaired ventilation re-

sponse to CO_2, after vagotomy, is therefore due to a relative inefficiency of the act of inspiration, and not to a reduced motor discharge. The inefficiency results from loss of the inhibitory inflation reflex and a consequent wasteful prolongation of the inspiratory discharge in each cycle.

3. After vagotomy an intermittent stimulation of one vagus, cutting short each inspiration, augments frequency of breathing and may augment minute volume. The total inspiratory discharge per minute is at the same time reduced.

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THE EFFECT OF GLYCOCOLL (GLYCINE) INGESTION UPON THE GROWTH, STRENGTH AND CREATININE-CREATINE EXCRETION IN MAN[1,2]

ALEXANDER S. CHAIKELIS

From the Biology Department, The City College, and the Department of Industrial Hygiene, Columbia University, Columbia Medical Center, New York

Boothby (1) reported that the onset of muscular fatigue can be delayed by the addition of a considerable amount of glycine to the diet of a normal individual. He also demonstrated that in cases of myasthenia gravis the feeding of glycine tends to restore the wasted muscle tissue, thereby indicating an effect upon the physiological state of these tissues. Wilder (2) believed that glycine affected the creatinine excretion following vigorous physical exercise. Subsequently Ray, Johnson and Taylor (3) presented their findings in which they noticed that when a given adequate amount of gelatine (60 grams) was added to the normal diet of men there was an invariable increase in the amount of work produced before fatigue set in. The results reported varied from 37 per cent to 240 per cent increase above the training level.

Since Boothby (1) found that normal subjects were unable to tolerate large amounts of glycine without some discomfort, and since gelatine as a substitute proved to be cumbersome in the study of a large series, where convenience and portability were prime factors, it was felt that the administration of glycocoll (glycine) in tablet form would be the most ready way of taking this substance. In addition to the above factors, the form in which the glycocoll was to be administered was motivated by the fact that, to the author's knowledge, none of the above papers, concerned with muscular fatigue and glycine, reported quantitative studies on a group of subjects sufficiently large for statistical tests of reliability. Furthermore, the absence of controls, in the above studies, was felt to be an omission of an important magnitude.

The ready availability of glycocoll in tablet form (Tablets Glycolixir-

Squibb) stimulated the undertaking of this study to determine what effect glycocoll (or glycine) had on growth (body weight, body height, and protoplasmic mass increase), upon endurance (ability to carry out sustained activity), on strength (muscular force), and upon the creatinine-creatine excretion following physical exertion in men.

EXPERIMENTAL.   The subjects who volunteered for this investigation were for the most part freshmen taking the prescribed course in Hygiene at The City College, and athletes from the Division of Physical Education of the Department of Hygiene at the College.   These subjects were males between the ages of 17 and 30.   The subjects were athletes and non-athletes, who may or may not have been in training.   Their normal daily routine was not modified in any respect, including diet, except in the partaking of the specific dosage of glycocoll or sugar.   The selection of the subjects to act as controls and as experimentals was by random sampling.

Observations were made on one series of controls, numbering 19 students, taking placebo tablets of glucose and lactose (2 grams each tablet) at the rate of six tablets per diem for the duration of the experiment. Observations were also conducted on two series of experimental subjects, numbering 20 students each, taking 1 gram of glycocoll in tablet form (Tablets Glycolixir-Squibb) at the rate of six tablets per diem for the duration of the experiment which was ten weeks.   It must be noted that the subjects were at no time aware as to whether they were taking glycocoll or sugar in as much as the tablets of glycocoll and the placebos were of the same size, shape, color, flavor, and were packaged the same way. The author was the only person who kept records as to the status of each subject.   Each series had its initial and terminal measurements recorded. These measurements totalled 14 individual items.   The grand total of observations on both the control series and the experimental series is 1632.

Tests and measurements.   Each subject was given the following tests and examinations before and after the experimental period of 10 weeks:

1. Height—measured to the nearest $\frac{1}{4}$ of an inch.
2. Weight—measured to the nearest $\frac{1}{2}$ pound.
3. Protoplasmic mass—calculated from the formula:

$$PT = 55 \text{ per cent } FW + 25 \text{ per cent } AW$$

where PT is the protoplasmic tissue or mass; FW is the fatless weight, according to table 8—Jones (4); and AW is the actual weight in pounds.

4. Pulse rate per minute was counted for an interval of 15 seconds.
5. Blood pressure (systolic and diastolic) in millimeters was taken with the Tycos sphygmomanometer.
6. The Barach Index—determined from the formula given by Barach (5):

$$(SP \times PR) + (DP \times PR) = K$$

where *SP* is the systolic blood pressure, *DP* is the diastolic blood pressure, and *PR* is the pulse rate per minute.

7. Rogers Strength Index—calculated from the formula given by Rogers (6) for the physical capacity test by omitting the measurement of lung capacity, which was considered not necessary, and by substituting the McCloy formula (7) for the determination of arm strength, because the factors of height and weight are more equitably taken care of in the computation. This Modified Rogers Strength Index is derived from the formula:

> Grip strength (rt. hand) in pounds + grip strength (lt. hand) in pounds + back lift in pounds + leg lift in pounds + (1.77 × weight of the body + 3.52 × no. of chins − 46) = K

The bracketted portion of the formula represents the McCloy arms strength formula.

8. McCloy Endurance Index. Since there are few good tests of endurance requiring no period of preliminary training, because the devisors of the tests have measured interchangeably endurance and strength, it was hoped that the McCloy Track Test would serve the purpose of testing the possible effect of glycocoll on endurance. This test consists of running a 60 yd. dash, and when completely rested (at the end of 20 min.) a 220 yd. dash at full speed. The endurance ratio is the time for the 220 yd. dash divided by the time for the 60 yd. dash. The slower the time the higher the index, while the lower the index the greater the endurance.

9. Total Creatinine—determined by collecting a 6 hr. sample of urine following the accomplishment of all the tests, and determining the creatinine-creatine excretion as total creatinine concentration per 100 cc. according to the method of Benedict (8) using the Pulfrich Photometer.

Procedure for the measurement of strength. In calculating the strength index the following steps were carried out in using the apparatus consisting of *a*, an oval hand dynamometer; *b*, a back and leg dynamometer; *c*, a horizontal bar for the measuring of chinning or pull-ups, and *d*, parallel bars for the recording of dipping or push-ups:

1. Grip strength. The subject places the rounded edge of the dynamometer against the palm, with the indicator also toward the palm. The subject was permitted to assume any position with the arm or body (which was noted for the retest at the end of the experiment's duration), so long as the hand or fingers did not rest against the body or any other object. The recording was noted to the nearest pound.

2. Back lift. In determining the back lift, the subject stood at attention with hands on the frontal surface of the thighs. The observer then hooked the handle into the chain so that the top of the bar was just below the tips of the subject's fingers. The individual then bent forward at the hips and grasped the bar at the ends with one palm forward and one

palm backward.   He, then, lifted steadily, but as vigorously as possible, and, after having exerted a maximum lift, released the pull slowly.   The observer recorded the lift in pounds.

3. Leg lift.   The position is the same as in the back lift.   The dynamometer bar was placed across the thighs in the obtuse angle formed by the thigh and the trunk.   The subject then was made to raise his head and chest and pull as hard as possible with legs and arms.   This lift was then recorded in pounds.

4. Dipping or push-ups.   The subject jumped to the cross rest with arms straight (this counted as one effort).   He then lowered his body until the angle of upper arm and forearm was less than 90°, and then pushed up to
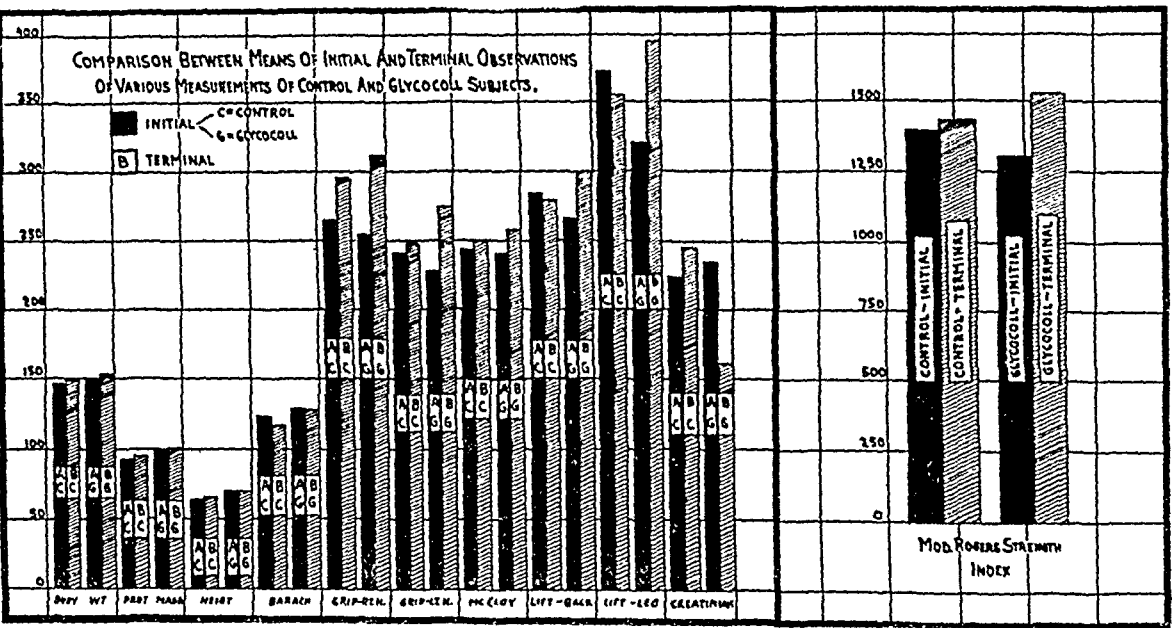
Fig. 1

the straight arm position.   He repeated this movement as many times as possible.

5. Chinning or pull-ups.   The subject's ability to raise and lower himself while hanging from a horizontal bar using the backward grip was recorded.   No kick, jerk, or a "kip" motion was allowed.   For both the push-ups and the pull-ups partial scoring was not included in the final calculations of the strength index.

ANALYSIS OF RESULTS AND DISCUSSION.   Figure 1 represents, in graphic form, the comparison between the means of the initial and terminal observations of both the control and the glycocoll series.   Table 1 summarizes the results obtained for each one of the 14 separate tests carried out on the control series of 19 subjects and the experimental glycocoll series of 40 subjects at the start and finish of the experiment.   The statistical analysis presented in this table is based on the following method of hand-

TABLE 1

Comparative analysis of the results obtained on a control series (19 subjects) and on an experimental glycocoll series (40 subjects), both series composed of normal, healthy, athletic and non-athletic young men of college age, with regard to each of 14 distinctive and separate tests

Control series: 19 subjects. Glycocoll series: 40 subjects

ANALYSIS	BODY WEIGHT (lbs.)	BODY HEIGHT (inches)	PROTOPLASMIC MASS (lbs.)	PULSE RATE PER MINUTE	BLOOD PRESSURE Systolic	BLOOD PRESSURE Diastolic	BARACH INDEX	GRIP-STRENGTH Right hand (lbs.)	GRIP-STRENGTH Left hand (lbs.)	LIFT-STRENGTH Back muscles (lbs.)	LIFT-STRENGTH Leg muscles (lbs.)	PUSH-UPS (DIPS)	PULL-UPS (CHINS)	MC CLOY ARMS STRENGTH INDEX	RUNNING 60 yd. dash (secs.)	RUNNING 220 yd. dash (secs.)	ENDURANCE INDEX	TOTAL CREATININE (CONC.) NINE MG. PER 100 CC.	MODIFIED ROGERS INDEX
Control series:																			
Mean (A) of initial observations	146.6	64.6	92.14	72.	110.	65.	126.	265.	241.	284.	367.	8.5	8.5	243.	8.5	32.5	3.84	226.	1400.
Mean (B) of terminal observations	148.4	64.8	93.47	71.	109.	67.	119.	294.	248.	281.	351.	8.5	9.6	250.	8.5	32.9	3.80	243.	1427.
Mean of the amount of change between initial and terminal observations	2.2	0.16	0.8	−1.0	1.	1.8	−1.6	29.	17.	0.3	−18.	0.0	1.0	7.3	0.0	0.4	0.01	17.	26.8
e° of mean change	2.7	0.2	0.9	4.5	3.2	4.4	7.9	34.9	53.0	29.6	50.1	1.2	2.2	11.6	0.8	0.6	0.1	35.2	69.9
e_M† of the mean change	0.633	0.045	0.211	1.063	0.760	1.045	1.873	8.216	12.022	0.966	11.805	0.272	0.512	2.737	0.199	0.138	0.027	8.300	10.482
Glycocoll series:																			
Mean (A₁) of initial observations	149.4	67.8	99.11	72.	110.	66.	130.	253.	229.	267.	322.	7.0	7.0	241.	8.7	32.9	3.80	235.	1312.
Mean (B₁) of terminal observations	152.7	67.9	100.20	71.	111.	68.	128.	310.	273.	299.	394.	8.0	9.0	256.	8.5	32.3	3.81	162.	1532.
Mean of the amount of change between initial and terminal observations	3.20	0.12	1.21	−1.05	0.25	1.00	−2.50	57.	53.	32.30	73.40	0.88	1.30	12.	−0.18	−0.57	0.005	−68.50	223.50
e° of mean change	3.7	0.1	1.1	7.7	5.0	4.5	16.3	37.5	27.6	44.3	63.1	2.0	1.7	9.5	0.3	1.1	0.1	44.9	119.1
e_M† of the mean change	0.859	0.034	0.247	1.720	1.120	0.997	3.054	8.382	6.186	9.916	14.113	0.440	0.373	2.129	0.050	0.246	0.030	10.054	26.040

Difference between means of change of Control and Experimental Series	1.00	0.04	0.41	0.05	0.75	0.80	0.93	23.00	36.00	32.00	91.40	0.88	0.30	4.70	1.03	-0.97	0.005	-85.50	196.70
†Standard error of the above difference	1.068	0.056	0.325	2.022	1.357	1.450	3.720	11.740	14.060	12.120	18.400	0.270	0.630	2.800	0.210	0.280	0.030	13.040	29.700
Critical ratio: Observed diff./Standard error	0.0	0.71	1.26	0.02	0.55	0.55	0.25	2.38	2.56	2.04	4.97	3.22	0.47	1.64	5.22	3.44	0.16	6.50	6.02
Chances of obtaining a difference equal to or greater than the observed difference due to errors of random sampling	46 in 100	24 in 100	10 in 100	49 in 100	48 in 100	48 in 100	40 in 100	57 in 10,000	52 in 10,000	41 in 10,000	0 in 10,000	0 in 10,000	32 in 100	5 in 100	0 in 10,000	3 in 10,000	44 in 100	0 in 10,000	0 in 10,000

* ϵ = mean deviation = $\sqrt{\frac{\Sigma d^2}{N-1}}$. See Scott (13).

† ϵ_M = mean deviation of the mean = $\frac{\epsilon}{\sqrt{N}}$.

‡ Standard error of the difference between means = $\sqrt{(\epsilon_{M_1})^2 + (\epsilon_{M_2})^2}$. See Chaddock (14).

ling the data: *a*, the amount of *change* in each test for each individual was determined; *b*, the mean of the algebraic sum of these changes for all the individuals together was then calculated; *c*, the difference between the mean change for the control series and the mean change for the glycocoll series was arrived at; *d*, the standard error of this difference was then determined; *e*, the critical ratio was then calculated; and, *f*, the probability was then determined from the statistical tables of Holzinger (9).

From Table 1 it is apparent that the controls increased their body weight by 2.2 lbs. which is equal to a percentage change of 1.50 per cent. The glycocoll group increased their weight by 3.2 lbs. above starting level for a change of 2.14 per cent. The experimental group as a unit did not show a statistically significant increase in body weight since the probability is only 46 in 100.

Changes in body height observed in both the control and the glycocoll series were responsible for the slight changes noted in the protoplasmic mass, but were not significant.

The Barach test frequently used to confirm the results of strength and endurance tests proved to have no significance in this research.

In the matter of the grip-strength test, using the right hand, the control series improved after 10 weeks to the extent of 10.94 per cent. The glycocoll series exceeded their initial level by 22.53 per cent after a similar period. The difference between the means of the degree of change of the control and experimental series is 28.0 lbs., and the chances of obtaining a difference equal to or greater than this observed difference due to errors of random sampling are 87 in 10,000.

As to the grip-strength test, using the left hand, the controls improved to the extent of 17.0 lbs. or an approximate 5 per cent while the glycocoll group improved to the extent of 23.14 per cent. The standard error of the difference between the control and glycocoll means of change is 14.06 and the critical ratio is 2.56. In this case the deviation exceeded by more than twice the standard error and may thus be formally regarded as significant.

The results of the lift-strength test when using the back muscles showed that whereas the controls improved to the extent of 0.3 lb. the experimental series improved to the extent of 32.3 lbs. This difference of 32.0 lbs. between the groups is significant because the chances of obtaining a similar or greater difference due to errors of random sampling are 41 in 10,000. When using the leg muscles the glycocoll series improved by 22.79 per cent. The difference between the control and the glycocoll series in this phase of the lift-strength test has complete significance in favor of the glycocoll series because the critical ratio was found to be 4.97, which is practical certainty.

The cross rest on the parallel bars is a familiar exercise to develop the

extensors of the elbow (triceps) and depressors of the humerus (latissimus, teres major, deltoid 3). Measuring push-ups or dipping on the parallel bars showed that the glycocoll series improved, and this improvement was statistically significant.

When a person hanging by his hands tries to lift his body with his arms he brings into play the flexors of the elbow (biceps, brachialis, brachioradialis, pronator teres) and the depressors of the humerus. The improvement of the glycocoll series over the control series to the amount of 0.3 was not statistically significant.

The McCloy arm strength test proved to be statistically not significant. The results as regards the modified Rogers Strength Index show that there is definite significance to the amount of improvement in the glycocoll series. In this case the critical ratio is 6.62 which is practical certainty.

No attempt was made to determine the total volume of urine voided during the 6 hour period immediately following the series of 14 tests. The total creatinine excretion represented as a concentration in milligrams per 100 cc. showed, in the control series, an increase of 7.52 per cent in the terminal determination at the end of 10 weeks. A very striking effect of glycocoll is in evidence here for the experimental series showed a remarkable drop in the quantity of total creatinine excreted after physical exertion. After 10 weeks' ingestion of glycocoll, the experimental series' total creatinine concentration dropped to extent of 68.5 mgm. per 100 cc. This change proved to be practical certainty beyond any peradventure of doubt when subjected to the statistical analysis, since the critical ratio obtained was 6.56.

In this study it was hoped that endurance would be demonstrated objectively in the McCloy endurance test. However, that test did not force the subject to extend himself to the limit of his "staying" ability, and so did not elicit any significant change due to glycocoll ingestion. Certainly a more elaborate test of endurance that involves no preliminary education and practice in its performance might prove more efficacious in measuring this phenomenon.

In this study no attempt has been made to ascertain the mechanism by which glycocoll effects this increase in muscular strength and the extensive retention of creatine. The analysis of all the results obtained by using the statistical method of amount of change indicates that glycocoll has some definite beneficial effects, which are quite significant in view of the fact that no dietary restrictions were imposed upon the subjects undergoing the test. Invariably these same subjects reported that, above all else, they observed, subjectively, a marked improvement in their ability to sustain for a prolonged period of time any form of physical activity of a rather strenuous character. No such apparent increase in the resistance to fatigue was reported by the members of the control series. If we accept

the fact that hydrolysis of phosphocreatine is an essential part of the complex reactions which accompany the combustion of carbohydrate in muscle, then this reaction appears to be influenced to a great extent by glycocoll. Tripoli and Beard (10) have demonstrated the creatinogenic action of glycocoll, and Beard and Pizzolato (11) later suggested that glycine could be methylated to sarcosine in the synthesis of creatine. Rose, Ellis and Helming (12) had already confirmed the concept of storage of creatine in the bodies of male subjects. Thus the production and storage of creatine, as a whole, tends to prevent extreme changes in the acidity of the internal environment of the muscle cell because the change from phosphocreatine to orthophosphoric acid involves the substitution of a weak acid for a strong one (15). Apparently this reaction permits the elaboration of greater amounts of lactic acid without any alteration of the reaction in the muscle.

SUMMARY

A group of 19 subjects, acting as controls and ingesting for a period of 10 weeks fixed quantities of sugar, was compared with a group of 40 subjects, acting as experimentals and ingesting for the same time interval 6 grams of glycocoll per diem, as to 14 distinct and different measurements devised to evaluate achievement of physical strength and endurance, and to measure the amount of total creatinine excreted in the urine during a period of 6 hrs. following the physical exertion in the performance of the tests.

Under the influence of glycocoll a number of the tests revealed results that were definite improvements over the initial efforts. The grip strength improved by 22.5 per cent and 23.1 per cent for the right and left hand respectively. The lifting strength improved by 12.0 per cent and 22.8 per cent for the back and leg muscles respectively. The total body strength measured as the modified Rogers strength index showed an improvement of 17.0 per cent. The total creatinine excretion showed a drop of 29.2 per cent as compared with the initial excretion level.

recording, as assistant observers, the results of the various tests performed by the subjects. To the many students who coöperated so earnestly in the experiment for the period of 12 weeks, the writer can only express his sincere appreciation and thanks.

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STUDIES ON CHOLINE ESTERASE ACTIVITY AND ACETYL-CHOLINE CONTENT OF THE CENTRAL NERVOUS SYSTEM

R. CORTELL, J. FELDMAN AND E. GELLHORN[1]

From the Departments of Physiology and Psychiatry of the University of Illinois, College of Medicine, Chicago

In spite of the fact that acetylcholine (ACh) affects the central nervous system (CNS) in minute quantities (Dikshit, 1934) the relation of functional changes in the CNS to the ACh content and the activity of the choline esterase (ChE) is not clear. Loewi (1937) reported that prolonged strychnine convulsions in frogs increased the ACh content of their CNS. Fegler and co-workers (1938–1939) found a decrease in total ACh in rabbit brain during strychnine convulsions, but a relative increase in free ACh. MacIntosh (1939) stated, however, that neither insulin hypoglycemia nor avitaminosis B had any effect on the ACh content of the brain. Pighini (1939) studied the ChE content of the brains of dogs and rabbits under conditions of tetanus intoxication and strychnine convulsions, but his data reveal no significant changes over the controls.

A simultaneous study of the ACh content and ChE activity of the CNS under a variety of conditions has been undertaken to determine possible changes in the concentration of either substance or in their relations to each other in states of altered brain function. The ACh content and ChE activity were determined in the brains of rabbits subjected to hypoglycemia, anoxia and metrazol, and in the brains of eserinized rabbits subjected to metrazol and strychnine convulsions. Further studies were made on the brains and spinal cords of frogs subjected to strychnine convulsions, heat coma and recovery from heat coma.

METHODS. For the determinations of ACh the brain tissue was cut up in trichloracetic acid, the extract shaken with ether, the water layer evaporated under vacuum, and assayed for ACh on the eserinized rectus abdominis of the frog (Chang and Gaddum, 1933). ChE was determined by grinding the tissue with NaCl and determining the enzymatic activity of the supernatant fluid by the continuous electrometric titration method of Glick (1937) using 0.002 N NaOH over a period of thirty minutes.

In the rabbit experiments the animals were killed by a blow on the head,

and the brain removed as quickly as possible. One half of the brain was used for the ACh determinations, the other half for the ChE determinations. Separate determinations were run on hemispheres and brain stems. In the frog experiments, two or three animals were pooled for the ACh determinations. The ChE determinations were made on single frogs. In both cases, separate determinations were run on brains and spinal cords.

The rabbits were unanesthetized. Anoxia was produced by inhalation of 7 per cent oxygen from Douglas bags (Gellhorn and Packer, 1940). Insulin[2] was injected subcutaneously; metrazol, eserine and strychnine were administered intravenously. In every instance, rabbits which received eserine were first injected with 1 mgm./kgm. of atropine intravenously.

TABLE 1

Choline esterase activity and acetylcholine content of rabbits' brain under conditions of insulin hypoglycemia and oxygen lack

NUM-BER OF ANI-MALS	TREATMENT	HEMISPHERE		BRAIN STEM	
		Choline esterase activity, cc. 0.002 N NaOH/g.	Acetylcholine content, γ ACh/g. tissue	Choline esterase activity, cc. 0.002 N NaOH/g.	Acetylcholine content, γ ACh/g. tissue
8	Control	21.74 (19.50–23.80)	1.5 (1.0–2.0)	29.36 (19.53–37.98)	2.3 (2.0–2.7)
6	Insulin, 0.5 U-/kgm.	20.27 (18.83–24.64)	1.3 (1.1–1.7)	31.91 (24.17–37.22)	2.1 (1.9–2.4)
3	Anoxia, 7%—2 hrs.	25.61 (22.97–28.00)	1.6 (1.4–1.9)	29.00 (23.46–34.35)	2.2 (2.1–2.4)
2	Insulin and metrazol (30 mgm.-/kgm.)	20.45 (19.73–21.07)	1.4 (1.2–1.5)	28.11 (27.52–28.70)	2.2 (2.1–2.2)

RESULTS. In the first series of experiments, six rabbits were made hypoglycemic by the injection of 0.5 U per kgm. of insulin, three rabbits were subjected to 7 per cent O_2 for two hours, and two rabbits were given 5U per kgm. of insulin followed 4 hours later by 50 mgm. per kgm. of metrazol. The values for ACh and ChE were compared with those of eight normal rabbits (table 1). In none of the experimental animals was there any change in the ACh content or ChE activity as compared with the controls.

A second series of experiments was run on eight rabbits subjected to eserine, two rabbits given eserine plus metrazol and four given eserine plus strychnine (table 2). Five of the eserinized rabbits were killed at

[2] Kindly supplied by Eli Lilly and Company.

the end of five minutes, and it was found that there was a marked suppression of ChE activity in both hemisphere and brain stem.   The ACh content of both parts of the brain was increased.   Three of the rabbits were killed after approximately an hour, at which time the ChE had returned to essentially normal values, while the ACh was still somewhat high. These experiments give proof that eserine injected into animals produces a reversible inhibition of ChE *in vivo*, accompanied by an increase in the ACh level.   Eserinized rabbits made convulsive with metrazol or strych-

TABLE 2

Choline esterase activity and acetylcholine content of rabbits' brain following intravenous eserine and eserine plus convulsants

NUM-BER OF ANI-MALS	TREATMENT	HEMISPHERE		BRAIN STEM	
		Choline esterase activity*	Acetylcholine content, γ*	Choline esterase activity	Acetylcholine content, γ
8	Control	21.74 (19.50–23.80)	1.5 (1.0–2.0)	29.36 (19.53–37.98)	2.3 (2.0–2.7)
5	Eserine, 0.25–0.75 mgm./kgm., 5 min.	10.68 (7.41–16.49)	2.5 (2.0–3.0)	12.74 (8.97–18.28)	3.0 (2.9–3.0)
3	Eserine, 0.75–1.5 mgm./kgm., 49 min.	18.69 (16.14–20.80)	2.1 (1.9–2.3)	26.55 (20.93–32.90)	2.8 (2.3–3.0)
2	Eserine, 0.5 mgm.-/kgm., 5 min.; metrazol, 30 mgm./kgm.	8.63 (7.94–9.31)	2.4 (2.1–2.6)	12.22 (11.76–12.68)	3.1
4	Eserine, 0.5 mgm.-/kgm., 5 min.; strychnine, 0.4 mgm./kgm.	10.40 (7.34–13.19)	2.9 (2.2–3.4)	11.25 (8.84–13.73)	3.2 (2.6–3.4)

* As expressed in table 1.

nine and killed after five minutes showed no differences in the ACh content or ChE activity of either hemisphere or brain stem from animals given eserine alone.

As there were no changes produced in the ACh content or ChE activity in the brains of warm blooded animals except in the experiments involving eserine, a third series of experiments was performed on frogs, which could be subjected to more prolonged changes in function of their central nervous system.   Excitation was produced by strychnine convulsions, which were allowed to continue for four hours.   Table 3 gives the results of these

experiments. Both the brain and spinal cord of strychninized frogs showed an increase in ACh content over the control frogs. The average value for the ChE activity was the same for both groups of animals. The results on the increase in ACh content confirm those of Loewi (1937).

TABLE 3

Choline esterase activity and acetylcholine content of frogs' central nervous system following prolonged strychnine convulsions

TREATMENT	Experiments	SPINAL CORD Choline esterase activity*	Experiments	Acetyl-choline content, γ*	Experiments	BRAIN Choline esterase activity	Experiments	Acetyl-choline content, γ
Control	12	87.18 (71.92–106.47)	6	4.5 (4.4–4.7)	8	40.83 (28.14–47.85)	5	5.8 (5.1–6.8)
Strychnine, SO$_4$, 0.1 mgm.	12	87.11 (66.67–117.58)	6	6.2 (4.8–7.1)	8	36.36 (28.79–51.67)	6	7.2 (6.5–8.5)

* As expressed in table 1.

TABLE 4

The effect of heat coma and recovery from heat coma on the acetylcholine content and choline esterase activity of frogs' central nervous system

TREATMENT	Experiments	SPINAL CORD Choline esterase activity, cc. 0.002 N NaOH/g.	Experiments	Acetylcholine content, γ ACh/g. tissue	Experiments	BRAIN Choline esterase activity, cc. 0.002 N NaOH/g.	Experiments	Acetylcholine content, γ ACh/g. tissue
Controls	12	87.18 (71.92–106.47)	6	4.5 (4.4–4.7)	8	40.83 (28.14–47.85)	5	5.8 (5.1–6.8)
Heat coma A*	5	36.76 (30.64–46.29)	4	3.1 (3.0–3.3)	5	19.31 (13.86–24.25)	5	4.5 (4.0–4.9)
Heat coma B†	4	60.81 (49.02–70.00)	3	5.4 (4.4–5.9)	4	31.52 (28.94–33.00)	3	5.9 (5.2–6.6)

* A, frogs subjected to 15 minutes of heat at 37–39°C. and killed immediately thereafter.

† B, subjected to 15 minutes of heat at 37–39°C., and killed two days later, when recovery had taken place.

In regard to the ChE activity, however, Nachmansohn (1938) reported that strychnine has a definite inhibitory action on ChE activity in *in vitro* experiments, and he calculated that the quantity of strychnine used to produce convulsions in frogs is sufficiently high to produce ChE inhibition *in vivo*, although he did not perform any such experiments.

Inhibition of activity of the central nervous system of frogs was produced by keeping the frogs at temperatures of 37 to 39°C. for fifteen minutes, thus producing a comatose condition. Analysis of the ACh content and ChE activity of the brain and spinal cord of these frogs showed a depression of ChE activity and a decrease in ACh content as compared to the control frogs (table 4). A second group of frogs was allowed to recover from the heat coma, and their CNS assayed two days later for ACh and ChE, at which time their reflexes were normal. The ACh content had returned to normal, and the ChE activity, although the values were higher than in the comatose frogs, had not quite returned to the control level.

DISCUSSION. Although these experiments offer no conclusive evidence for or against the rôle of ACh in the transmission of impulses across the synapses within the central nervous system, they show that in the warm blooded animal the esterase may be inhibited *in vivo* by 50 per cent or more, with consequent increase in the ACh content of the central nervous system without grossly disturbing its function. Furthermore, they demonstrate that in cold blooded animals (possibly on account of the more severe procedures?) varying degrees of central excitability are associated with similar and reversible changes in the ACh content of the nervous system.

SUMMARY

1. Experiments on unanesthetized rabbits show that neither prolonged anoxia, hypoglycemia nor convulsions alter the acetylcholine content and choline esterase activity of hemispheres and brain stem.

2. Intravenous injection of eserine (0.5 mgm./kgm.) markedly inhibits choline esterase activity and increases the acetylcholine content of the brain. The effects are reversible with time.

3. Neither convulsions nor oxygen lack alter the acetylcholine and the choline esterase activity in eserinized rabbits.

4. Experiments on frogs show that strychnine convulsions are accompanied by a marked increase in the acetylcholine content of the brain and spinal cord. There is no significant alteration in the choline esterase activity.

5. When frogs are warmed to 38°C. or higher, a comatose condition appears. The central nervous system of such animals shows a decrease in both acetylcholine content and choline esterase activity. When the heated frogs are allowed to recover, they show, 48 hours later, together with restoration of nervous system functions, a return of choline esterase activity and acetylcholine content to approximately normal values.

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TRANSFER OF RADIOACTIVE SODIUM ACROSS THE PLACENTA OF THE GUINEA PIG[1]

LOUIS B. FLEXNER AND HERBERT A. POHL

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Md., and the Department of Anatomy, the Johns Hopkins University

A considerable number of investigators (15, 17, 19) interested in problems related to placental permeability have emphasized that in this field there is an almost complete lack of quantitative data. This is due to the absence in the past of a suitable method of investigation. Observations on the placenta of a preliminary nature (7, 8) with radioactive sodium have demonstrated that the use of isotopes provides the direct and relatively simple approach demanded by the problem.

The present project has a double goal. The first is to study transfer across the placenta throughout the gestation period, so far as is feasible, and to correlate the resulting data with important related phenomena such as the growth rate of the fetus. The second is to widen these studies to include the several morphological types of placentae. In the end, consequently, it is hoped to have established a foundation for the comparative physiology of placental transfer.

Radioactive sodium ion, present as NaCl, has been chosen as the beginning tracer material for several reasons. It is easily prepared by deuteron bombardment of NaCl, it has a favorable half life, its behavior in the body is not complex, and it meets the requirement of being a strictly physiological substance.

APPARATUS AND METHODS. A. *Apparatus for measurement of radioactivity.* In the present work a pressure-ionization chamber (1) coupled to a Wülf or Edelmann type single-fiber electrometer (21) was used. The apparatus is moderately inexpensive, quite rugged, highly precise, and sensitive.

In practice the radioactive material is placed in a cup at a geometrically reproducible position below the pressure-ionization chamber (fig. 1). The pressure-ionization chamber consists of a brass chamber of large volume (about 1500 cc.) with a window at its base made of aluminum foil 0.1 mm. thick. It is filled with N_2-gas under 25 lbs.' pressure. Within

the chamber is a space enclosed by a charged, wire screen maintaining a potential gradient of roughly 40 volts per centimeter across the space to the central collecting rod. Radiation from the radioactive material produces ions in its path. The positive ions so created, and within the space enclosed by the high-potential screen, are moved by the potential gradient to the central collecting rod which is insulated with amber. As the ions are deposited the insulated rod becomes charged, and the charge spreads over a shielded lead to the platinum-coated quartz fiber or string of the electrometer. (Platinum-coated quartz fibers have been found superior to Wollaston wires in the Wülf electrometer.)
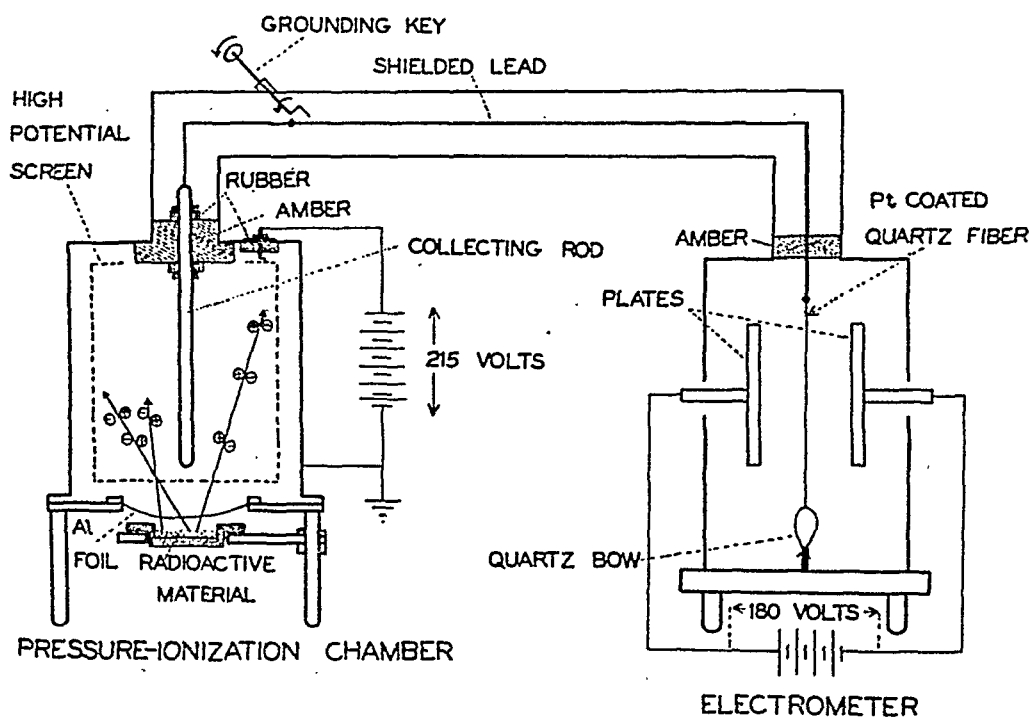
Fig. 1. Schematic diagram of pressure-ionization chamber and electrometer circuit.

The quartz fiber is suspended between two plates. Established between these plates is a potential gradient of about 180 volts per centimeter so that the fiber moves between these plates when it acquires a charge. The rate of movement of the fiber is observed through a microscope with a micrometer eyepiece and timed with a stopwatch. Since the charging rate, the strength of the radioactive sample, and the rate of movement of the fiber are all directly proportional, it is sufficient to measure the rate of movement to determine the sample's strength. The instrument is always calibrated with a uranium standard. A grounding key serves to shunt off the charging current to the fiber when the apparatus is not in use.

There are a number of electrometers and vacuum tube circuits (4, 6,

21) which have been proposed for use with an ionization chamber. The Dersham and Lindemann electrometers possess a peculiar and unexplained drift effect peculiar to the needle torsion type instrument (14). Because of this, these electrometers have a scale deflection which varies considerably from linearity with charging rate and makes this type of instrument undesirable for the purpose at hand (7 separate instruments were tested).

The electrometer circuit is very sensitive to temperature change and consequently the apparatus is placed in a constant temperature box.

Measurement of radioactivity with the pressure-ionization chamber and string electrometer presents the following sources of error:

1. Non-linearity of the electrometer response when plotted against sample strength. This is minimized by careful construction of the electrometer, particularly with respect to the amber insulation and quartz fiber, and by using the electrometer, at constant temperature, within ranges safely below its point of instability.

2. Geometric variability. Unless successive positions of the samples relative to the ionization chamber are exactly comparable, considerable error may be introduced. In this work care was taken to distribute liquid and ash samples evenly over the bottom of a special paper-lined bakelite cup. The cup was then placed in a mechanical mount which assured a reproducible position of the sample relative to the window of the ionization chamber at time of measurement.

3. Vibration of the fiber. This was minimized by providing a firm mounting for the instrument.

4. Battery voltage fluctuations. These are important only in the electrometer circuit and at high sensitivity. Here the sensitivity doubles with voltage increments of the order of 0.05 volt. This is a variation of $\frac{0.05 \text{ volt}}{180 \text{ volts}}$ or 2.5 parts per 10,000 in the voltage. Therefore accurate thermostatic control and careful use of the batteries is necessary.

5. Statistical fluctuations. Rate measurements involving low numbers of particles entering the chamber are affected by the statistical fluctuation of the numbers of particles entering the chamber. If a precision of "ϵ" in the expression of the rate is desired, at least $\frac{1}{\epsilon^2}$ particles must be counted, e.g., if $\epsilon = 1$ per cent, $\frac{1}{\epsilon^2} = \frac{1}{(0.01)^2} = 10,000$ particles must be counted in order to state the rate with a probable error of not more than 1 per cent.

6. Background. This is the minimal deflection rate of the instrument and is due to stray radiations entering the ionization chamber, to battery voltage fluctuations, etc. It is rather constant in magnitude and must be accounted for in the deflection rate read during measurement of a radioactive sample. In our apparatus, with its 1500 cc. chamber, background

.is equivalent to a beta-ray source emitting 15 betas per second in all directions and placed at the standard position occupied by a sample.

7. Absorption of the radiation. Absorption by the samples is variable and must be accounted for. This has conveniently been done with the aid of experimentally established correction curves (fig. 2) relating the amount of the material to its absorption. The volume of liquid samples (plasma) taken for measurement was always 2 cc.; the correction factor for these samples was 1.32.

8. Radioactive decay. This may be corrected by means of the equation for radioactive decay:

$$\log\left(\frac{N}{N_0}\right) = \frac{-0.3010\ t}{\lambda}$$

where N/No is the fraction of radioactive atoms left after time t and λ is the half life of the particular radioactive material.
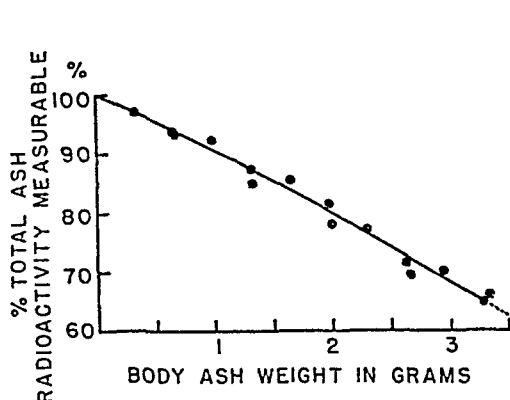
Fig. 2

Fig. 3

Fig. 2. Absorption of radiation from known quantities of Na^{24} by varying amounts of body ash of rats. The cup containing the ash had a diameter of 5 cm.
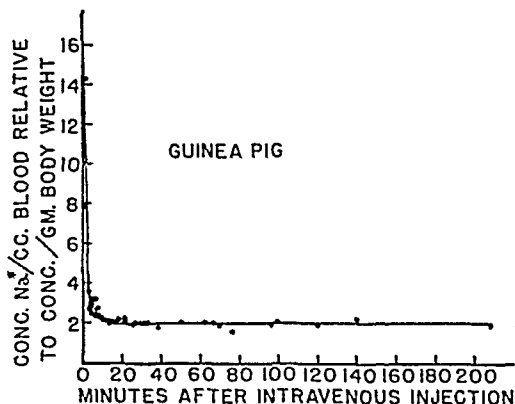
Fig. 3. Rate of equilibration of Na^{24} (injected intravenously) with the body fluids.

9. Adsorption. It was found that the bakelite cups retained appreciable activity of samples placed in them, even after careful washing. The problem was answered by the use of replaceable waxed paper linings for the cups.

10. Miscellaneous errors. These may arise from contamination of samples or measuring apparatus with radioactive materials, especially those of long half life, accidentally spread in the laboratory; from transfer losses in handling the samples; and from such inaccuracies as are present in timing the interval of fiber movement, evaluating readings on the micrometer scale, etc.

The overall precision of the radioactivity measurements attained with usual sample strengths (100 to 2000 counts per second) is about 4 per cent,

but in rare cases where samples are only a fraction of background (3 to 5 betas per second) the precision may be no more than 40 per cent.

Preparation of samples. Radioactive sodium (Na[24]) was prepared by means of the electrostatic pressure generator of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, as previously described (8). The sodium chloride targets were used for an experiment at least 24 hours after preparation to allow time for the disappearance of any contamination of radioactive Cl[38].

About 40 mgm. of NaCl dissolved in 2 cc. of warm water were used for each of the experiments. This salt had an activity of between 1 and 2 microcuries in all the experiments except those where fetal size was very small; in these occasional instances, as high as 5 microcuries were used. The maximal doses used were well below radiation tolerance limits (5,11,18).

Units. Activity of the radioactive material is expressed in terms of beta-particles per second, e.g., a sample of strength 100 beta-rays per second in our apparatus would be one that gave a deflection rate equivalent to a beta-ray point source emitting 100 beta-rays in all directions and situated at the standard measuring position in our apparatus. One microcurie is about 37,000 particles per second in these units.

All the results on rate of transfer of Na[24] to the fetus have involved measurement of the radioactivity of the fetus and a corresponding sample of maternal plasma. For comparison of the results of the different experiments, the concentration of Na[24] found in the fetus has been divided by that found in 1 cc. of maternal plasma. The term, "corrected," frequently used in presentation of the results therefore means that the amount of Na[24] found in the fetus has been corrected to a concentration of one beta-particle per cubic centimeter maternal plasma. These units will be adhered to in subsequent papers. To change data on transfer of Na[24] across the cat placenta, given in a preliminary report (7), to this basis, they must be multiplied by two, as they were based on a unit concentration of one beta per 2 cc. plasma.

Procedure with animals. Nine experiments were performed to follow changes with time in blood concentration of Na[24] after intravenous injection. Large, adult guinea pigs were chosen. Under nembutal anesthesia, a cannula was introduced into the proximal end of the carotid artery. At zero time, Na[24] in isotonic solution containing heparin was injected into an arm or leg vein. Samples of blood of 1 or 2 cc. were then taken from the carotid cannula and measurement made of their radioactivity.

Pregnant guinea pigs, totaling 30, formed the bulk of the experimental material. They were obtained from several independent sources and so were not of a uniform strain. The experimental procedure with all of these animals was alike. They received an intravenous injection of radioactive

salt under light ether anesthesia. In the rat (8) such anesthesia has been found to be without demonstrable effect on placental transfer. Anesthesia was immediately discontinued after the injection. After a time interval fixed by the purpose of the experiment, the animal was again etherized, the fetuses delivered by Caesarian section, and immediately thereafter a sample of heart's blood taken from the mother. The fetuses were weighed with a precision of about 5 per cent after removal of the fetal membranes (with fetuses of low weight several were pooled), ashed at red heat with sulphuric acid, the ashes weighed, and the radioactivity of the maternal blood plasma and ashed fetal remains then determined. The placenta was separated from the decidua basalis and weighed after superficial blood had been absorbed by filter paper.

RESULTS. *Rate of escape of Na^{24} from blood plasma.*[2] The concentration levels of Na^{24} in the blood relative to that in a unit body weight at various periods after intravenous injection are shown in figure 3. Curves representing the fall in blood Na^{24}-concentration with time were obtained for nine animals. To secure a clear, averaged picture of events the several curves were fitted on a common graph (fig. 3) by multiplying each by a factor which served to bring their equilibrium values in close agreement. It is to be expected that within a short interval following injection, thorough mixing of Na^{24} with the blood did not take place and consequently the Na^{24}-concentrations at these times lose precision in their meaning. For example, assuming blood volume to be 10 per cent of body volume, the initial concentration of Na^{24} per cubic centimeter blood relative to that per gram body weight would be equal to 10 units on the graph. A value as high as 14 has, however, been found one minute after injection.

For the present purposes, the important observations are that the concentration of Na^{24} in the blood falls to a value within 90 per cent of its equilibrium value in 5 minutes, and remains constant for over 3.5 hours afterwards. This is in substantial agreement with the findings of Hevesy (12) on the rabbit.

The data of figure 3 can be used to calculate the volume of total extracellular fluid. Such a calculation gives the result that the extracellular fluid is 25 per cent of the weight of the animal on the assumption that sodium is distributed only in the extracellular fluid, and that plasma volume is 50 per cent of total blood volume. This value compares favorably with that found by the same (9) or analogous methods (20).

Establishment of equilibrium between maternal plasma and fetus. In order to plan experiments to determine the rate of transfer of Na^{24} across the placenta, it was necessary to know the shape of the curve describing the establishment of equilibrium between fetus and maternal plasma. In these experiments, all members of a litter were delivered as nearly

[2] The symbols Na^{24} and Na are used for the respective ions.

simultaneously as possible; obvious changes in the state of the uterus made any other procedure unacceptable. This observation was substantiated by the finding that delayed second deliveries made 30 minutes or more after the first gave placental transfer rates as much as 70 per cent lower than in the undisturbed uterus.

The data of figure 4 indicate that equilibrium is reached in fetuses of 60 grams or greater in from 5 to 7 hours. The concentration of Na^{24} in the fetus appears to increase linearly for 2 or 3 hours after intravenous injection into the mother. Measurements of the increase in concentration of Na^{24} up to this time consequently form a reliable criterion of the rate of transfer of Na^{24} across the placenta. In view of these findings, the routine
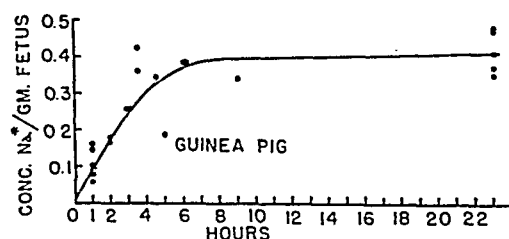
Fig. 4
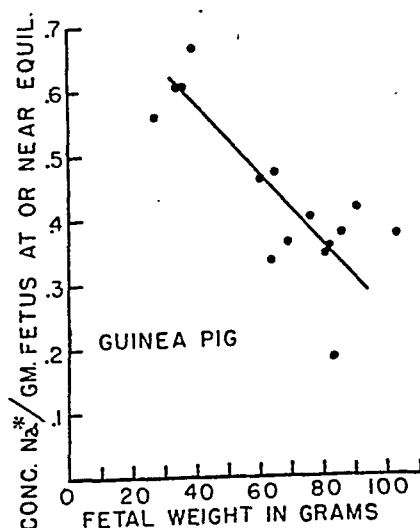
Fig. 5

Fig. 4. Rate of equilibration of Na^{24} in the maternal plasma with that of the fetus. All of the fetuses for these experiments weighed more than 60 grams. The concentration of Na^{24} in the fetus has been corrected to a concentration of one beta-particle per cubic centimeter maternal plasma as explained in the section above on "units."

Fig. 5. Variation of equilibrium concentration of Na^{24} with fetal size. The concentration of Na^{24} in the fetus has been corrected to a concentration of one beta-particle per cubic centimeter maternal plasma.

procedure for determination of placental transfer rates has been to remove fetuses 1 hour after injection of Na^{24} into the maternal circulation.

The magnitude of the concentration of Na^{24} reached at equilibrium is a function of fetal size, being greater in small than in large fetuses. This is shown in figure 5. From these equilibrium values, the approximate volume of extracellular fluid in fetuses at various stages of development can be calculated on the assumptions that the placenta does not secrete Na and that the Na^{24} is equally distributed throughout the extracellular fluid and is limited to it. Multiplying the equilibrium values (corrected) at each stage by 100 gives the extracellular fluid weight as per cent of the

body weight. This is 25 per cent for a fetus near term and 60 per cent for a 30 gram fetus.

Rates of placental transfer. The experiments have been planned to measure the rates of placental transfer at different parts of the gestation period. In all experiments measurement has been made of the concentration of Na^{24} in the maternal plasma and of the total quantity of Na^{24} in the fetus (each sample was taken at known interval, about one hour, after intravenous injection into the mother); and of the placental and fetal weights. Such measurements can be expressed in several ways as required to analyze the results from one or another aspect, e.g., in terms of total transfer to a fetus per unit time, of transfer rate per unit weight fetus, or of transfer rate per unit weight placenta. The data necessary for deriving these relations are presented graphically. Figure 6 gives the data on changes in placental weight with fetal weight (fetal weight can be translated into gestation age by reference to fig. 8). The change in total hourly transfer of Na^{24} to the fetus with change of fetal size is presented in figure 7.
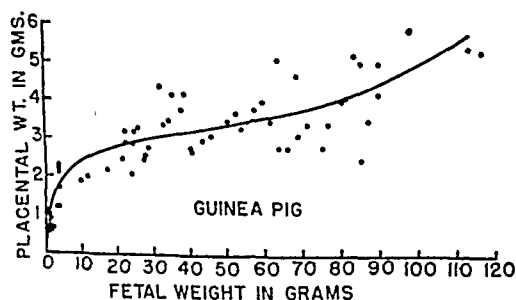
Fig. 6
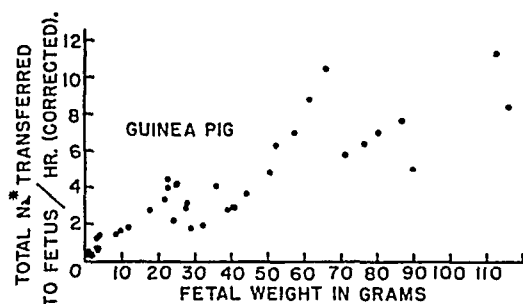
Fig. 7

The data of figures 6 and 7 have been used to construct the curves of figures 8 and 9. In figure 8 are shown the changes in rate of transfer across a unit weight of placenta throughout most of intra-uterine life. As is clear from the curve, the transfer rate per unit weight placenta makes a striking overall increase with age. Thus at 28 days the transfer rate is about 0.2 unit; at 46 days, 1.4 units, and at 62 days, about 1.9 units. Transfer of Na^{24} across a unit weight of the 62-day placenta is consequently about 10 times as fast as across a unit weight of the 28-day placenta. The rise in transfer rate with fetal age does not appear to be continuous, however. An apparent minimum occurs at a gestation age of about 50 days and there is evidence of a decrease as term is neared. The indicated minimum at 50 days is determined by the findings on 3 litters, totaling 9 fetuses.

Figure 9 gives the rate at which Na^{24} is supplied from the maternal plasma to each gram of fetus as this rate varies with fetal age. This rate is high in the early fetus and falls with increase of fetal age. Thus in the 28-day fetus about 0.5 unit Na^{24} is transferred per gram fetus per

hour; in the 40-day fetus, about 0.16 unit and in the 62-day fetus, 0.1 unit. Figure 9 also shows the daily per cent weight increase of the fetus during the greater part of the gestation period (the daily per cent weight increase at any point is 100 times the slope of the tangent to the age-weight curve at that point). This has been calculated from the data of Ibsen as quoted
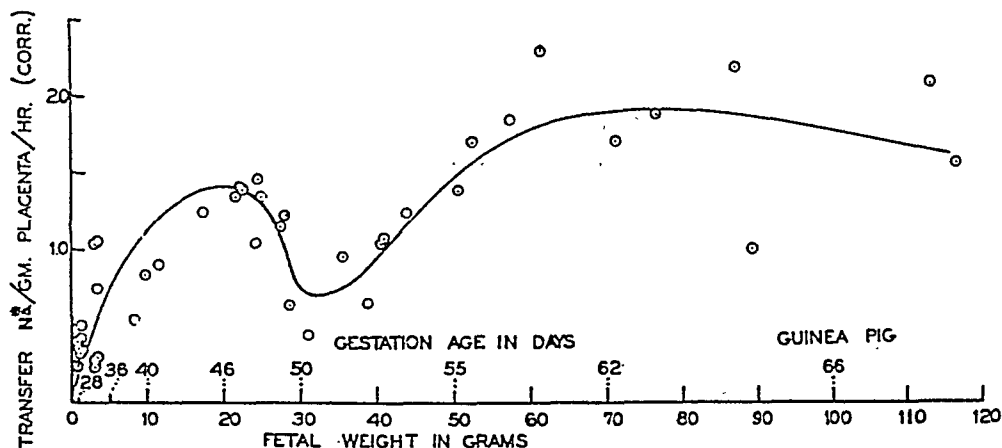
Fig. 8. (The gestation age has been estimated from the data of Ibsen (13).)
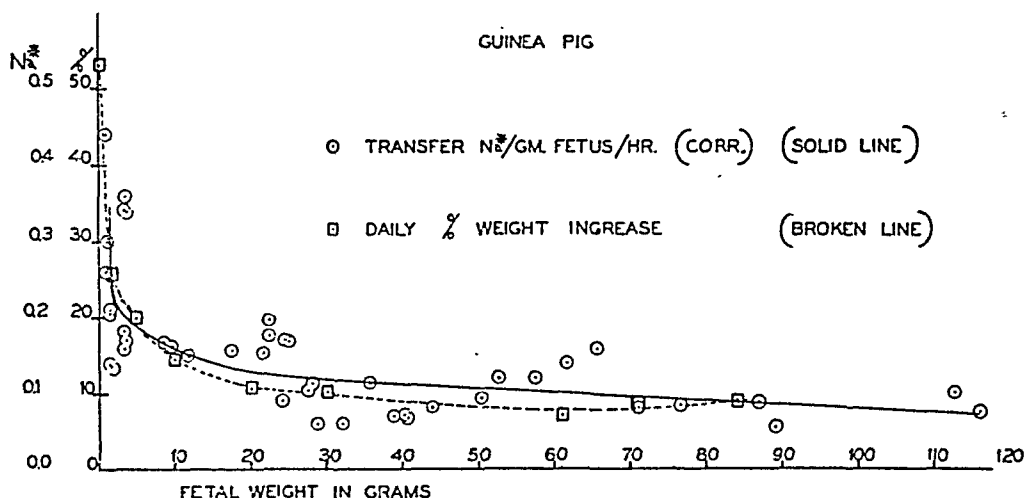
Fig. 9

by Needham (19). It is apparent from figure 9 that the parallelism of these 2 curves is striking.

Fetal need for Na relative to supply across placenta. By using the transfer rate of Na^{24}, the equilibrium concentration of Na^{24} and the hourly per cent growth rate of the fetus, it is possible to calculate by just what factor the supply of Na exceeds the need. The ratio of the supply of Na to the amount of Na retained by the fetus will be designated the safety factor.

The safety factor is readily calculated. The Na^{24} present in the maternal circulation is thoroughly mixed with all the Na of the maternal plasma in a fixed proportion. It may consequently be assumed that any Na^{24} which passes from the mother to the fetus is accompanied by Na from the mother and in the same proportion, provided no separation of isotopes occurs. Hence the Na supplied to a unit weight of fetus per hour is directly proportional to the amount of Na^{24} supplied to it per hour. On the other hand, the hourly accretion of Na by this growing unit of tissue is a direct function of the hourly weight increase of the tissue and of the amount of Na per unit weight of tissue. This last, the amount of Na in a unit weight of tissue, is directly proportional to the concentration of Na^{24} per gram reached by the fetal tissue when at equilibrium with the maternal plasma. The safety factor is then:

Transfer Na^{24} per gram per hour (corr.) $\times$ 24 hrs. $\times$ 100 $\div$ equilibrium concentration Na^{24} per gram (corr.) $\times$ daily per cent weight increase. The value of the safety factor for guinea-pig fetuses is given in table 1.

TABLE 1

FETAL WEIGHT	TRANSFER Na^{24} PER GRAM FETUS PER HOUR (CORR.)	DAILY PER CENT WEIGHT INCREASE	EQUIL. CONC. Na^{24} PER GRAM FETUS (CORR.)	SAFETY FACTOR	TOTAL Na TRANSFERRED TO FETUS PER HOUR	TOTAL Na RETAINED IN HOURLY GROWTH OF FETUS
gms.					*mgm.*	*mgm.*
100	0.08	10	0.27	65	26	0.4
30	0.12	10	0.63	50	12	0.25
3	0.20	22	(0.75)	30	2	0.07
1	(0.45)	33	(0.8)	(40)	1.5	0.04

The data of columns 2 and 3 have been interpolated from figure 9; the data of column 4, from figure 5. Values in parentheses have been extrapolated.

The quantity of Na transferred to the fetus per hour as well as that incorporated in new growth can be separately calculated by assuming a reasonable value for the concentration of Na in the maternal plasma. If the total Na and total Na^{24} transported to the fetus per hour be designated respectively by Na_F and Na^{24}_F, and the concentration of Na and Na^{24} in the maternal plasma, by $Na_{M.P.}$ and $Na^{24}_{M.P.}$; then:

$$Na_F = Na_P^{24} \times \frac{Na_{M.P.}}{Na_{M.P.}^{24}}$$

The expression on the right is simply the total Na^{24} transfer per hour (corrected) multiplied by the concentration of Na in the maternal plasma.

The hourly accretion of Na by the growing fetus is: $Na_{M.P.}$ $\times$ equilibrium concentration Na^{24} per gram (corr.) $\times$ fetal wt. $\times$ daily per cent weight increase $\div$ 24 $\times$ 100.

The quantities of Na transferred per hour and retained in hourly growth are given in columns 6 and 7 of table 1. Their ratio is the safety factor. They have been derived from the data of figure 7, assuming a concentration of 3.3 mgm. Na per cubic centimeter of maternal plasma.

DISCUSSION. In the stages of gestation studied here, an increase of over a hundredfold in fetal weight is accompanied by only about a twelvefold increase in placental weight (fig. 6). The gain in placental weight which accompanies fetal growth gives, however, an entirely misleading concept of the change in rate of placental transfer as measured by Na^{24}. An equally important factor is the change which occurs in transfer rate per unit weight of placenta. The experimental findings indicate that the rate of transfer across a unit weight of placenta increases about 10 times from the early to the late stages of pregnancy in the series. It is to be noted that these values apply only to transfer to the fetus proper and do not include fetal blood in the placenta.

The placenta, then, is adapted by changes in its size and in its unit transfer rate to the size and so to the nutritional requirements of the fetus. How good is this adaptation in the guinea pig as measured by Na^{24}? Perfect adaptation from point of view of fetal growth would mean that at those periods of relatively rapid growth, where there is a large demand for substances out of which to build new tissue, there would be a correspondingly rapid transfer across the placenta. An examination of the relative growth curve of the fetus at different periods of pregnancy shows it to be similar to the curve describing the change of rate of transfer to a unit weight of fetus during the gestation period. The transfer rate per gram fetus is high in early pregnancy, despite the low transfer rate per unit weight placenta, because of the large size of the placenta relative to fetal size. The results suggest the hypothesis, wholly tentative, that the fundamental principle underlying change in placental transfer during the gestation period is that placental transfer to the fetus shall parallel the growth rate of the fetus.

It is impossible to give a quantitative explanation of the change in transfer rate from the maternal circulation to the fetus proper found with change of intrauterine age. The magnitude of total transfer to the fetus will depend upon factors which apply both to the decidua basalis and to the placenta. For a particular substance these factors are: the area of surfaces across which transfer occurs, the rate of blood flow past these surfaces, the nature of the tissues separating the two circulations, and the chemical state of the substance being transferred. The increase in weight of the placenta (fig. 6) may be taken as evidence of a purely qualitative kind that the effective surface area increases on the fetal side as gestation progresses. The weight of the decidua basalis reaches its maximum about two-thirds of the way through pregnancy and then slowly declines (13).

There are no observations known to us on the rate of blood flow through the placenta or decidua basalis of the guinea pig. However, direct observations have been made on the rate of blood flow through the placenta of the sheep (3) and through the maternal portion of the uterus in the pregnant rabbit (2); these at best may be considered no more than suggestive for the guinea pig. The findings on the sheep seem to indicate that the rate of flow in the capillaries of the placenta may double during the last fifth of pregnancy. The degree to which this increase in blood flow will increase transfer rate depends upon its effect upon the concentration gradient between the two circulations. Measurement of the rate of blood flow through the vessels of the pregnant uterus of the rabbit during the last half of pregnancy gives evidence which suggests that during this period the rate of blood flow through the decidua basalis does not change considerably.

Of clearer meaning for interpretation of increase in placental transfer are the changes which occur with intra-uterine age in the tissues separating the maternal and fetal circulations. The placenta of the guinea pig, in the classification of Grosser (10), is of the hemochorial type, i.e., throughout the bulk of the placenta, the tissues separating maternal from fetal circulations are entirely fetal and consist of chorionic epithelium and endothelium of fetal blood vessels. With progress of gestation, however, the chorionic epithelium thins (10, 16) and finally so largely disappears that Mossman (17) has suggested the term hemoendothelial for this type placenta. These histological findings fit the conclusion that increase in total transfer as well as increase in transfer rate per unit weight placenta both depend in part upon the thinning of the membranes placed between the fetal and maternal circulations.

SUMMARY

1. A simple pressure ionization chamber-string electrometer circuit for measurement of radioactivity is described.

2. *a.* The guinea-pig fetus comes to within 10 per cent of equilibrium with Na^{24} in the maternal plasma in from 5 to 7 hours. This compares strikingly with the extracellular fluid of the mother which comes to within 10 per cent of equilibrium with the plasma in about 5 minutes.

b. Large changes take place in the apparent proportion of fetal extracellular fluid to fetal body weight throughout gestation.

3. Changes in rate of placental transfer of Na^{24} per unit weight placenta have been measured from the twenty-eighth day of pregnancy until term. The transfer rate of Na^{24} increases about 10 times during this period.

4. The relative growth curve of the fetus is parallel to the curve describing the change of rate of transfer of Na^{24} to a unit weight of fetus at different periods of pregnancy.

5. The fetus receives across the placenta, throughout the stages of development studied here, an average of about 50 times as much Na as is incorporated in the growing tissues.

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REFLEX STUDIES AFTER MUSCLE TRANSPLANTATION[1]

W. G. WATROUS AND J. M. D. OLMSTED

From the Division of Physiology, University of California Medical School, Berkeley, California

Muscle transplantation, i.e., the translocation of the tendinous insertions of muscles, has been used with a great degree of success on human patients. Although every skeletal muscle cannot be transplanted and then perform its new function in the proper way, many cases are on record where the muscles do behave in the expected manner; to do this may require an extensive period of learning if an antagonistic action is conferred (Legg, 1923). Experiments on the lower animals, however, have given uniformly negative results. Taylor (1935) tried to convert the gastrocnemius of a frog into a flexor of the ankle, but was without success. Sperry (1939) crossed the ankle flexors and extensors in rats and even after 10 months' time could find no evidence of reversion of response in ordinary activities or in special trained performances. Others have transplanted supernumerary limbs or muscles in toads and rats and in each instance activity of the transplanted muscles is "homologous," e.g., is similar to that of the corresponding normal muscles and is thus indicative of an inviolable predetermination of response which in some instances may be harmful to the well-being of the animal.

Similarly the insertion of an eye muscle is often shifted in the human patient so as to make that muscle more effective or less so through changing its mechanical advantage. For example, the superior oblique has been substituted for a paralyzed internal rectus, with both cosmetic improvement and restoration of binocular vision (Wiener, 1928; Peter, 1933). More striking still are the experiments of Marina (1912, 1915), who found complete interchangeability of all the eye muscles of monkeys; within four days after crossing the externus and the internus behind the eyeball, convergence and divergence, nystagmus, voluntary movements and cortically-induced movements were claimed to be normal. Dusser de Barenne and de Kleyn (1928) crossed the internus and externus of rabbits and obtained conflicting results. Where the nystagmus was normal it was found that the retractor bulbi muscle was well developed; in other

[1] This investigation was supported by a grant from the Research Board of the University of California.

rabbits, the nystagmus remained reversed up to a year's time.   Neither monkeys nor man, however, possess the retractor bulbi muscle.   Olmsted, Margutti and Yanagisawa (1936) believed that the return of normal movement subsequent to crossing of the superior and external recti of cats and dogs was due to a process of central relearning, but unpublished observations by the present authors over a three year period have shown that after complete removal of all the extrinsic muscles of the dog's eye, except the retractor bulbi, there follows a gradual regaining of a limited ability to move the eye in all directions, particularly in those dogs whose retractor bulbi is well-developed.

In order to determine whether any possible reorganization of the central nervous system to meet such altered conditions becomes fixed so that the new responses are truly reflex, we have attempted to convert flexor muscles of the limbs into extensors and vice versa in adult cats and dogs, and the superior oblique into an adductor in the rabbit.

In different cats we have tried the following transplants: fastening the tendon of peroneus longus, a flexor, subperiosteally to the calcaneus a short distance behind the ankle joint; sewing flexor digitorum longus to the tendon of Achilles; sewing the tendon of soleus to the tendon of peroneus longus.   In the dog we have fastened either tibialis posticus or peroneus longus—both flexors—to the calcaneus.   The majority of these operations were unsuccessful since, although the limb was immobilized in a plaster cast for some days after the operation, the new attachments failed to hold.   Five operations, however, were successful, and 27 to 126 days were allowed for readjustment to the new situation.   The animals were then decerebrated and the isolated reflex activity of the transplanted muscle was contrasted with originally or newly synergic muscles, or with the original antagonist; comparison was also made with the same muscle on the control leg.   Various ipsilateral and contralateral sensory nerves were stimulated electrically to produce the reflex responses.

Without exception the transplanted muscles under the influence of reflex activation behaved as they would have done had transplantation not been performed.

The tracing on the left of figure 1 is the record of the reflex contraction of soleus, an extensor, in a cat in which this muscle had been successfully placed in a flexor position, and of its normal antagonist, tibialis anticus, which should now be its synergist.   At the time of sacrifice there was no incoördination in the walking of this animal so that from external appearances it might have been inferred that readjustment of the soleus to its new function had been perfected.   However, upon decerebration typical exaggerated tone appeared in soleus in spite of its flexor position, and stimulation of the ipsilateral posterior tibial nerve caused inhibition of this tone.   The same reciprocal relation of the transplanted soleus with

tibialis anticus that normally occurs in this pair of muscles is well shown in this record.

The central part of figure 1 shows the reflex responses of a dog's flexor muscle, peroneus longus, after being transposed to an extensor position. There was no increase in its tone on decerebration. Stimulation of the ipsilateral popliteal nerve is here seen to result in a co-contraction with its normal synergist, tibialis anticus, which should now be acting as its antagonist. Stimulation of the contralateral popliteal nerve, instead of causing contraction of the transposed peroneus longus, resulted in inactivity of it and of its normal synergist as shown in the right hand part of figure 1.
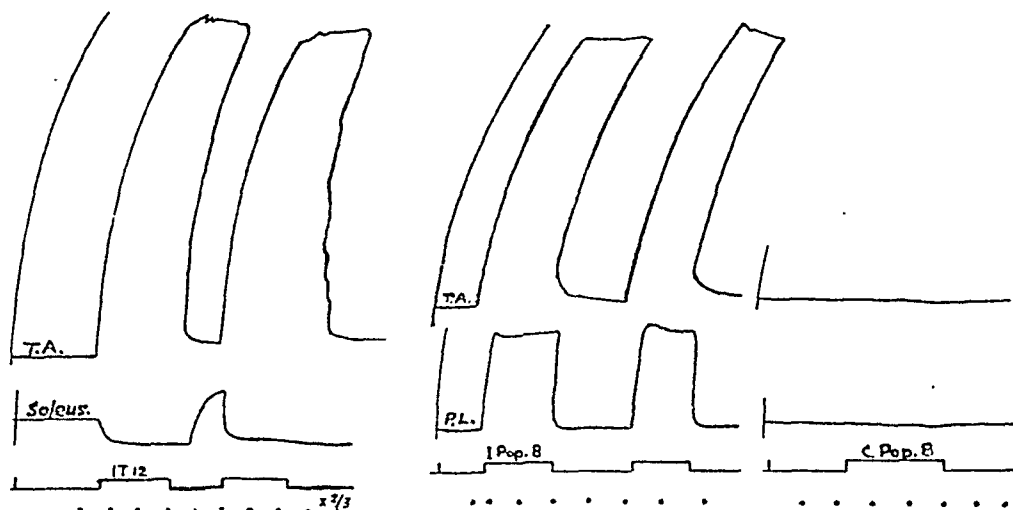
Fig. 1. At left: reflex response of tibialis anticus (flexor) and soleus (extensor) of a cat—the latter muscle transposed to the flexor position—to stimulation of the ipsilateral tibial nerve, showing undisturbed reciprocal innervation. In center: reflex response of tibialis anticus (flexor) and peroneus longus (flexor) of a dog—the latter muscle transposed to an extensor position—to stimulation of the ipsilateral popliteal nerve, showing undisturbed co-contraction. At right: reflex response of tibialis anticus and transposed peroneus longus of a dog to stimulation of the contralateral popliteal nerve, showing inactivity of both muscles.

In preparation for the final experiments on the rabbits, a one-stage preliminary operation for intracranial section of the oculomotor nerve was performed (Hines, 1931). Approximately a month was allowed for convalescence to be sure that paralysis of the internal rectus muscle was complete; it was noted that the nictitating membrane remained protruded after paralytic symptoms had set in and that the internal quadrant of the retractor bulbi muscle could still adduct the eye to a slight extent (cf. Dusser de Barenne and de Kleyn, 1928). This was shown by the definite though small inward movement of the eyeball when the rabbit was rotated in a horizontal plane. The superior oblique was then freed beyond the trochlea and sewed to the eyeball above the insertion of the internus in

such a way that there was no slack when the eyeball was in the primary position.   Approximately three months were allowed for readjustment.

Three types of control were used: 1, rabbits in which the third nerve had been cut intracranially and the eye muscles left untouched; 2, others in which the superior oblique was transplanted and the third nerve left undisturbed; 3, entirely normal animals, except one whose skull had been trephined as in preparation for cutting the third nerve.

At the time of the final experiment, the procedure was the same for the control and experimental series: the rabbit was decorticated under ether anesthesia; the superior oblique and external rectus muscles were freed from the eyeball, and tied to silk threads which later could be attached to very light isotonic bell-crank levers.   As many as possible of the remaining muscles were completely removed from the orbit by means of a tonsil snare.   A cord was tied around the optic nerve and ophthalmic artery and the eyeball was completely removed.   The rabbit was then placed on a turn-table which could be rotated in a horizontal plane.   A Czermak head holder fixed the head in position; it was necessary to fix tightly a brass tube into the orbit in order to render the orbital fascia immovable; and the thorax and legs were tied down.   The levers wrote upon a horizontally-placed kymograph mounted upon the turn-table.

Records were made of the rotational- and after-nystagmus due to rotation in clockwise and counterclockwise directions.   With these precautions there was scarcely any artefact, so that markings on the drum were very little influenced by movements other than contraction of the muscles in question, particularly during after-nystagmus.   The significant feature was the slope of the lines indicating the direction of the slow and fast components of nystagmus.   These movements were found to be similar in every respect to those recorded by de Kleyn (1925) from the corresponding eye muscles of normal rabbits during caloric stimulation.   It was noted that although the slope of the lines was the same, the base line might change, indicating a change in tone of both the superior oblique and external rectus, during rotation in addition to purely phasic responses.

Since the performance of the superior oblique exactly paralleled that of the external rectus in all cases, it was evident that in spite of transplantation, there was no remodulation in the central nervous system.   This was true even in those rabbits in which the internal rectus was functional and might be supposed to be able to facilitate adjustment of the transposed oblique in assuming the new function.

The fact that transposition of muscles in these experiments had in no wise altered the original reflex pattern of response, together with the difficulty experienced in making the transplanted limb muscles hold in their new positions may be considered as supporting Weiss' (1936) theory of "homologous response."   Weiss has described experiments on amphibia

which purport to show that muscles possess some biochemical factor, a "modulus" which "specifies" the activity of the nervous centers by way of the peripheral nerves.   The modulus is supposed to have control over the activity of the motoneurones in the simple behavioral pattern of the amphibia.   Hence, if a muscle remains connected to the same nerve centers regardless of where it has been transposed, it would have to respond in a predetermined fashion.   A corollary of this theory would demand that each different muscle must have a different modulus.   An experiment by Sperry (1939) on rats is interpreted in this light.   The failure to produce alteration in reflex pattern of response in our experiments argues that there is some mechanism which prevents the development of a response proper to the new conditions.

SUMMARY

When tested by reflex activity the isolated responses of translocated muscles of the hind limb of the adult cat and dog and of the translocated superior oblique of the rabbit show no alteration in function, even when as long as four months is allowed for readjustment.

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THE EFFECTS OF DENERVATION AND OF STIMULATION ON EXCHANGE OF RADIOACTIVE POTASSIUM IN MUSCLE

T. R. NOONAN, W. O. FENN AND L. HAEGE

From the School of Medicine and Dentistry, The University of Rochester, Rochester, New York

The experiments which we wish to report represent an attempt to use radioactive potassium to test the theory that stimulation is accompanied by an increase in muscle permeability. We did find evidence of increased penetration of the labelled potassium but this result, in all probability, was chiefly due to an increased circulation while the effect which is due to muscular contraction *per se* would seem to be relatively small or absent.

Measurements of the permeability of muscle to injected radioactive potassium have shown that the penetration into muscle is complete in rats in about 4 hours (Noonan et al., 1940). During the first one or two hours after injection therefore an excellent opportunity presents itself of studying variations in the speed of penetration which can be caused by various procedures.

METHODS. These experiments were performed chiefly on rats anesthetized with urethane. Muscles were stimulated either by applying electrodes directly to the sciatic nerve which was exposed as far centrally as possible or by inserting a needle electrode through the skin in the region of the sciatic notch and stimulating the nerve without further operation. After stimulating for 1 hour, or in control experiments after denervation for a period of an hour, the animal was killed by bleeding and matched muscles from the two hind legs were taken for determination of the radioactivity. For samples we usually used the gastrocnemius group, the tibialis group and sometimes the semimembranosus. Muscles were dissolved in concentrated nitric acid in tared weighing bottles. The volume of the digest was determined by weighing and by an independent determination of the specific gravity. A 3 ml. sample of the digest was put into a Bale ionizing chamber (immersion type) and measured with a Geiger-Müller counter (Bale et al., 1939).

CALCULATIONS. The radioactivity (RA) and the potassium radioactivity (KRA) are calculated as follows:

$$RA = \frac{\text{counts found per kgm. of tissue}}{\text{counts injected per kgm. of body weight}}$$

$$KRA = \frac{RA \times 100}{\text{mM potassium per kgm. of tissue}}$$

$$\text{Per cent penetration} = \frac{KRA \text{ of tissues} \times 100}{KRA \text{ of plasma}}$$

Since the differences in RA between paired muscles in the rats were relatively large compared to the possible differences in potassium content due to stimulation we have not usually gone to the trouble of analyzing for potassium and calculating KRA. Since, however, the potassium content of rat muscle is known to be about 113 mM per kgm. (Fenn and Cobb, 1935), the KRA (previously called "potassium activity", Noonan et al., 1940) may be found approximately by multiplying the RA by $\frac{100}{113}$. In the frog experiments the differences in RA due to stimulation were less marked and we have, therefore, analyzed all the frog muscles for potassium.

RESULTS. The results of 6 different types of experiments on rats are shown in table 1. The figures represent the radio activities (RA) of the muscles. The results show that without a single exception the experimental muscles contained a higher concentration of the radioactive potassium than the control muscles. In rats 1 and 2 the muscles of one side were stimulated while those of the other side were left intact and resting as control. The penetration was on the average 5 times as rapid on the stimulated side. The difference was in the same direction but only twice as large as the control value in rats 3, 4 and 5 where the electrodes were applied directly to the peripheral end of the cut sciatic. Repeating the same experiment with rat 6 but with the control nerve cut and not stimulated did not change the result, the penetration still being about 5 times as rapid on the stimulated side. It seemed possible that simply cutting the nerve might permit sufficient vasodilatation to increase significantly the rate of penetration. In rat 7, therefore, the control nerve was cut while on the experimental side the nerve was stimulated without being cut. Here the penetration was 1.5 times more rapid on the stimulated intact side than on the denervated control side. Thus if the circulation increase is the determining factor it can be concluded that stimulation and the consequent accumulation of metabolic products causes a greater increase in the circulation than mere denervation. That denervation by itself does increase the rate of penetration, presumably by increasing the rate of circulation, is shown in rats 8 and 9 where the experimental nerve was cut while the control nerve was intact, neither side being stimulated. In these experiments denervation alone doubled the rate of penetration. Finally in rats

TABLE 1

Penetration of radioactive K into stimulated and resting rat muscles

PROCEDURE	RAT NUMBER	MUSCLE	RADIO ACTIVITY	
			Exper.	Control
Both nerves intact. Exper. side stimulated by needle electrode inserted through skin	1	g	0.91	0.13
		t	1.03	0.09
		b	0.82	0.11
	2	g	2.36	0.75
		t	2.68	0.36
Average..................................			1.56	0.29
Experimental nerve cut and peripheral end stimulated. Control nerve intact	3	g	1.55	1.07
		t	1.49	1.13
		(r*	0.27	1.18)
	4	g	1.19	0.98
		t	1.41	0.42
	5	g	1.34	0.65
		t	1.58	0.51
Average..................................			1.42	0.78
Both nerves cut. Experimental nerve stimulated	6	g	0.60	0.15
		t	0.69	0.09
		s	0.57	0.14
Experimental nerve intact and stimulated. Control nerve cut	7	g	1.60	1.12
		t	1.88	1.11
		s	1.12	0.69
Experimental nerve cut. Neither nerve stimulated	8	g	1.09	0.25
		t	0.92	0.13
		(r*	0.38	0.32)
	9	g	1.38	0.90
		t	1.44	1.03
		b	0.88	0.43
Average..................................			1.14	0.55
Control nerve cut 4 days previously. Rat exercised for 1 hour after injection of radioactive potassium	10	g	0.76	0.60
		t	1.55	1.17
	11	g	1.17	0.77
		t	1.24	0.89
Average..................................			1.18	0.86

Abbreviations: g = gastrocnemius and soleus, t = tibialis and peroneus, b = biceps femoris, s = semimembranosus, r = rectus femoris.

* Not stimulated or denervated and not included in the averages.

10 and 11 one sciatic nerve of each rat was cut under ether anesthesia. Four days later, without further anesthetic, 2 cc. of a 2.6 per cent solution of radioactive potassium chloride were injected intraperitoneally into each rat. Rat 10 was then made to swim for an hour in a water bath at 30°C. while rat 11 continued the normal exercise of cage life for a similar period. At the end of an hour both rats were killed. On the average the penetration was 1.4 times as fast in the exercised muscles of both rats.

In a few of the experiments of table 1 values were also obtained for the potassium content of the muscles and for the radioactivity and potassium content of the plasma. Thus the potassium radioactivities of both muscles

TABLE 2

Potassium contents and percentage penetration in rat muscles

EXPERI-MENT	MUSCLE	POTASSIUM IN mM PER KGM.		PLASMA KRA	TIME AFTER INJECTION	PENETRATION	
		Exper.	Control			Exper.	Control
					hours	per cent	per cent
3	t	108	113				
	g	115	125				
	(r	119	119)				
4	g	88	108	1.48	1.3	91	56
	t	98	81	1.48		97	36
5	g	70	130	1.56	1.5	122	32
	t	93	128	1.56		109	25
6	g	91	114				
8	g	168	124	2.15	1.0	31	10
	t	120	114	2.15		36	6
	(r	125	116	2.15		14	13)

Experiments correspond to rats of same number in table 1.

and plasma could be calculated, the ratio between them being the per cent penetration of the radioactive potassium into the muscle or the per cent of the muscle potassium which had exchanged with the plasma potassium. The values for the potassium contents in these experiments (table 2) show that (with one exception, 4t) the stimulated muscle contained less potassium (per gram wet weight) and the denervated muscle contained more potassium than the control muscles. This is in agreement with previous results (Fenn and Cobb, 1935; Fenn, 1937).

The percentage penetration shown in the last two columns of table 2 indicates that both denervation (no. 8) and stimulation (nos. 4 and 5) markedly increased the rate of exchange. Indeed in rats 4 and 5 the ex-

change in the stimulated muscles appears to be practically complete in 90 minutes while their control muscles had exchanged only about one-third of their potassium in the same period. In this respect stimulated muscle behaves like diaphragm and heart both of which are continuously active and both of which may show potassium radioactivities greater than the simultaneous values (of KRA) in the plasma 1 or 2 hours after injection.

It seems probable that urethane anesthesia retards slightly the penetration into muscle. The average penetration into the control muscles of table 2 is 27 per cent in 1 hour. In our previous experiments with non-anesthetized rats taking only such exercise as is incident to normal cage life we found an average penetration of 47 per cent in 1 hour in 6 experiments (Noonan, Fenn and Haege, 1941).

Values given for the rectus muscle (in brackets, tables 1 and 2) in two experiments may be regarded as control experiments since this muscle was not stimulated and not denervated by treatment of the sciatic nerve. The potassium contents were not significantly changed by the procedure but in one stimulation experiment (no. 3) the radio activity of the rectus muscle was much diminished on the stimulated side compared to the control side as if the stimulated muscles of the same leg had received an increased blood supply at the expense of the rectus.

These experiments show that an increase in circulation such as may be caused by denervation may double the rate of penetration of radioactive potassium. This finding throws some doubt upon the interpretation of the effects of stimulation. The increased rate of penetration which is observed must be due in part at least to an increase in the circulatory rate. By experiments on rats we have not been able so far to rule out circulatory effects and have no evidence therefore that the muscle cell becomes any more permeable when it is stimulated.

In the hope of excluding complicating circulatory effects we turned to some similar experiments with isolated frog muscles. Pairs of muscles were carefully dissected out and divided in matched sets between two vessels each of about 20 ml. capacity and containing 6 ml. of Ringer's solution. Platinum electrodes were sealed through the walls of each of the vessels, emerging inside under the surface of the Ringer's solution. Thus the muscles of one set could be stimulated at will while remaining immersed in the Ringer's solution. Stimulation at just "maximal" intensity was continued for about 1 hour with a series of twitches delivered at a frequency of about 1 per second. At the end of the stimulation period the muscles of the stimulated side were removed from the solution, gently blotted, weighed and digested in nitric acid. The digest was analyzed for potassium and for radioactivity. The results of 2 experiments in table 3 show the final weight (in per cent of the initial weight), the number of counts, the micromols of K per gram of final weight and the counts per micromol of K (the

latter is similar to KRA but is uncorrected for the amount injected). The KRA of the muscle in per cent of the KRA of the solution is given in the last column. It represents the percentage of the total potassium of the muscle which has exchanged with the radioactive potassium of the solution and is therefore the per cent penetration. Of the 5 pairs of muscles studied 3 show slightly more and 2 show slightly less penetration on the stimulated side. On the average therefore there was no effect which could be attrib-

TABLE 3

Penetration of K into stimulated and resting frog muscles in vitro

Two experiments

MUSCLE	STATE	WEIGHT	POTASSIUM	COUNTS PER GRAM	COUNTS PER μMK	PENETRATION
Experiment 12. Stimulate 1 hour at 66 per minute						
		per cent of initial	μM *per gram*			*per cent*
Sartorius	Stim.	113	71.9	4680	65.1	11.3
	Rest	101	80.6	4330	53.7	9.4
Semitendinosus	Stim.	107	70.2	5200	74.1	12.4
	Rest	99	83.4	6310	75.6	13.2
Solution			3.48	2000	574	
Experiment 13. Stimulate $1\frac{1}{2}$ hours at 60 per minute						
Sartorius	Stim.	98	75.0	1534	20.5	14.0
	Rest	96.5	78.6	1553	19.8	13.5
Semitendinosus	Stim.	111	74.8	1385	18.5	12.7
	Rest	99.5	74.7	1350	18.1	12.4
Peroneus	Stim.	107	71.4	699	9.8	6.7
	Rest	99.5	78.1	816	10.5	7.2
Solution			3.42	500	146.2	

S = stimulated, R = resting. Potassium and counts are calculated per gram of final wet weight. Muscles used were sartorious, semitendinosus, ileofibularis and peroneus.

uted to stimulation. This would suggest that the effect observed in the rat muscles was entirely due to the secondary effects of muscular activity upon the circulation.

In considering these experiments of table 3 it may be observed that in every case the stimulated muscles gained water in comparison to the controls. It is probable that part of this increase in weight was due to extracellular water. If this had been corrected for by deducting the appropriate

amounts of potassium and of counts from the totals found in the muscle the counts per micromol of K so calculated for the muscle fibers would have been even less on the stimulated side. In every case the potassium per gram of final weight was less on the stimulated side but this was partly due to the increase in weight on that side. When the potassium is calculated on the basis of the initial weight only 3 of the 5 pairs show a loss of potassium, 1 shows no change and 1 an increase. The counts per micromol of potassium are, of course, the same for both methods of calculation.

Two other experiments with frog muscles are reported in table 4. In the first of these (no. 14) radioactive potassium was injected into the dorsal

TABLE 4

Penetration of K into resting and stimulated frog muscles in situ

MUSCLE	STATE	WEIGHT	POTASSIUM	COUNTS PER GRAM	COUNTS PER μMK	PENETRATION
Experiment 14. With natural circulation. Stimulate 1 hour at 66 per minute						
		mgm.	μM per gram			*per cent*
Gastrocnemius	Stim.	991	74.2	451	6.1	9.4
	Rest	860	82.0	455	5.6	8.6
Semimembranosus	Stim.	644	76.0	550	7.2	11.2
	Rest	552	87.2	542	6.2	9.6
Rectus	Stim.	1462	74.8	635	8.5	13.1
	Rest	1343	80.8	640	7.9	12.3
Plasma			7.86	508	64.6	
Experiment 15. Perfused frog. Stimulate 15 minutes at 114 per minute						
All on hind legs	S	3581	58.5	1910	32.7	10.8
	R	2904	72.4	2465	34.0	11.3
Plasma—arterial			3.74	1130	302	
Venous before stim.			3.50	932	266	
Venous after stim.			5.51	890	162	

lymph sac and peritoneum of a frog. Twenty-five minutes later the brain was destroyed by a clamp, one sciatic nerve was exposed in the abdomen by an incision in the back, and was stimulated 66 times per minute for an hour. At the end of this time blood was drawn by syringe (with heparin) from the aorta and samples of plasma and hind leg muscles were taken for counting and for K analysis. The muscles of the stimulated side contained uniformly more water and less potassium per gram of wet weight than their controls and a slightly greater fraction of their K had exchanged with radioactive plasma K. This small difference, however, may have been due to an increase in extracellular fluid with potassium radioactivity equal to that of the plasma. If half of the increase in weight of the stimulated

muscle over the control muscles were extracellular this would approximately explain the increased penetrations observed. This experiment, therefore, offers no evidence of increased permeability of muscle on stimulation. Apparently also there was no increase in circulation on the stimulated side. In general the penetration into frog muscle is slower than in rat muscle.

In experiment 15, table 4, the hind legs of a frog were perfused with 3 per cent acacia Ringer's solution plus 10 per cent of washed beef red cells. The venous perfusate was collected from a cannula in the abdominal vein, the renal portal flow being tied off. The potassium in the solution was radioactive. Stimulation of one sciatic nerve at 114 shocks per minute was begun 20 minutes after the flow started and was continued for 15 minutes, after which the muscles were removed for analysis for K and radio K. Venous samples were collected for analysis for 2 minutes before and for the first 3 minutes after stimulation began. The rates of flow measured for these 2 samples were 2.08 and 2.12 ml. per minute respectively. There was, therefore, no increase in flow in the active muscle unless there was a corresponding decrease in the control muscle. All the muscles on each leg were pooled for analysis. The stimulated muscles were obviously wetter, weighed 1.24 times more than their controls and contained less total K and less radio K. The percentage exchanged was if anything less than on the control side. If correction could be made for the extracellular fluid the calculated penetration on the stimulated side would have been even less. This experiment also suggests, therefore, that in perfused frog muscle there is no increase in permeability due to stimulation.

The analyses of the perfusate are of special interest in this experiment. Figures for the counts per gram show that the muscles removed radioactive K from the perfusate both before and after stimulation. Before stimulation the muscles removed a small amount of total K but after stimulation a large increase in the K content of the perfusate was observed. From these figures it is possible to calculate the amount of radio K exchanged for normal K for comparison with the net transfer. Let $C =$ counts per gram, $K =$ micromols of K per gram and subscripts t, a and v refer to tissue, arterial plasma and venous plasma respectively. Let $x =$ micromols of K per gram which exchange.

Then $C_v = C_a +$ counts which come in $-$ counts which go out

$$= C_a + x\left(\frac{C_t}{K_t} - \frac{C_a}{K_a}\right) - \frac{C_a}{K_a}(K_a - K_v)$$

Now $\frac{C_t}{K_t}$ is the KRA of the tissue which is unknown but it must have been

very low because at the end of the experiment it was only 34 as compared to $302 = \dfrac{C_a}{K_a}$ in the artery. If $\dfrac{C_t}{K_t}$ increases linearly during the perfusion its value at the time when stimulation began may be estimated at about 5 since only $\frac{1}{7}$ of the total volume perfused had been collected at this time. Considerable variations in this estimate will not change the calculation very much. Before stimulation, therefore, the following values may be substituted

$932 = 1130 + x\,(5 - 302) - 302\,(3.74 - 3.50)$, whence $x = 0.42$ micromol of K exchanged per cubic centimeter of perfusate. A similar calculation after stimulation gives a value of 0.93 μmol exchanged $\left(\text{using } \dfrac{C_t}{K_t} =\right.$

20 in place of C_a/K_a in the last term $\Big)$. This apparently indicates an increase in the amount exchanged as a result of stimulation, but the error in the radioactivity is such that even this large difference is not significant. The calculation does show, however, the order of magnitude of the exchange which occurs in one capillary transit in addition to the net transport of K.

DISCUSSION. Only in the experiments with frog muscles in vitro has it been possible to rule out possible increases in penetration due to improvements in the circulation. Since under these conditions no increase in permeability was found as a result of prolonged stimulation it is logical to conclude that in the case of rat muscles contracting in situ the increased penetration which was observed was due entirely to an increase in the capillary bed or to an increased rate of flow. From experiments on rats alone this conclusion is not justified although it can be shown clearly that the rate of penetration can be increased by the vasodilatation which follows immediately after denervation.

Our conclusions relative to frog muscles agree well with those of Dean (1940) who observed that over a period of 1 hour the penetration of radioactive K into frog muscle was increased only from 10.6 per cent to 11.2 per cent by substituting nitrogen for oxygen. Thus the penetration seems to be uninfluenced by rather drastic changes in the treatment.

<div align="center">SUMMARY</div>

1. The rate of penetration of radioactive K into rat muscles in 1 hour is increased as much as 5 fold by stimulation and about 2 fold by denervation.

2. In isolated frog muscles stimulated in Ringer's solutions containing radioactive K no increased penetration could be observed. This conclusion was confirmed by stimulation of frog muscles in the body both with natural circulation intact and during perfusion.

3. The experiments indicate that muscular activity does not increase the permeability to potassium, all the increase observed in rat muscles being attributable to an increase in the circulation.

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THE RESPONSE OF THE ADRENALECTOMIZED DOG TO RENIN AND OTHER PRESSOR AGENTS

J. W. REMINGTON, W. D. COLLINGS,[1] H. W. HAYS, W. M. PARKINS AND W. W. SWINGLE

From the Section of Physiology, Biological Laboratory, Princeton University, Princeton, N. J.

Most investigators are agreed that although a moderate hypertension can be developed in an adrenalectomized dog with kidneys rendered ischemic, it is not maintained unless supportive therapy in the form of salt or cortical extract is given (1–6). Usually, but not always (7), the elevated pressure has been reduced to normal or subnormal levels by the time definite symptoms of adrenal insufficiency have appeared. The pressor effect of the ischemic kidney is presumably through the action of renin, which induces a generalized vasoconstriction without influencing directly the heart rate or blood flow (8–11). It has been reported (12–13) that the blood pressure response to renin is greatly diminished in the adrenalectomized animal, a diminution not correlated with either symptoms of insufficiency or the level of the blood pressure, but only with the time interval after supportive therapy is withdrawn. A comparable reduction in response to tyramine, epinephrine or amphetamine was not observed. A direct interrelationship between the hormone or hormones of the adrenal cortex and the sensitivity to renin is therefore indicated. Our interest in the adrenal cortex and in renin led us to investigate further this possible interrelationship.

METHODS. The renin used in these experiments was prepared by the method described previously (14). Two general dosage levels were used. The first was large enough to insure the maximum pressor response of the test animal. The extract used yielded on assay (15) a 40 mm. Hg rise in mean pressure with a dose of 0.1 mgm. per kgm. body weight. A total of about 3 mgm. renin of this potency, in a 10 kgm. dog, will evoke the maximum pressor response (16). In these experiments a dose of 9.5 mgm. was used, which is roughly 3x maximum. The second dosage level employed was sufficient to produce a rise of 30 to 40 mm. Hg in the intact dog.

The responses of adrenalectomized dogs to the lower renin dose were compared to those evoked by epinephrine (adrenalin hydrochloride,

Parke, Davis and Co.), pitressin (Parke, Davis and Co.), and barium chloride, at dosage levels selected to yield a rise of about 30 mm. Hg in mean pressure.

Sixteen healthy adrenalectomized dogs, table trained for blood pressure recording without anesthesia, were used in these experiments. Six of these were given the overdosage of renin, four the low renin dosage, three, epinephrine and barium chloride, and three, pitressin. The time sequence of the testing was selected to minimize any precipitating influence the response to the pressor agent, *per se*, might have had on the normal course of adrenal insufficiency. For example, with the large renin doses, only one test was made in one cycle, extract being restored immediately after

TABLE 1

Protocol of an adrenalectomized dog run through several cycles of insufficiency to determine changes in the pressor response to overdosages of renin

DATE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	RED BLOOD CELL VOLUME	HEMOGLOBIN	BLOOD UREA-N	BLOOD PRESSURE	RISE IN PRESSURE	PEAK PRESSURE	REMARKS
	m.eq./l.	m.eq./l.	m.eq./l.	per cent	grams per cent	mgm. per cent	mm. Hg	mm. Hg	mm. Hg	
3/6							97	70	167	Unilateral
3/10							106	70	176	Unilateral
3/27	140.1	113.8	5.1	36.3	13.5	19.9	96	76	172	Maintenance extract
4/2	133.5	100.6	6.1	41.8	13.1	31.2	68	56	124	Insufficiency
4/8	139.7	112.8	5.0	27.1	10.2	19.4	90	72	162	Normal—on extract
4/15	133.7	107.2	6.4	29.9	13.1	46.9	54	45	99	Insufficiency
4/22	142.7	111.8	5.2	22.0		18.6	95	70	165	Normal—on extract
4/23	141.7	117.9	5.3	22.2	8.4	18.8	95	71	166	Off extract—24 hours
4/27	135.1	108.8	5.8	23.7	8.5	22.3	79	83	162	Insufficiency
5/3	130.3	106.4	8.4	33.1	11.1	86.1	64	50	114	Severe insufficiency

the test. For a complete series of pressor responses at different stages of adrenal insufficiency, each animal therefore went through repeated cycles of insufficiency and return to normal.

RESULTS. I. *Overdosage of renin* (table 1, fig. 1). Control tests on seven unanesthetized intact dogs with the 9.5 mgm. dose of renin ranged from 57 to 77 mm. Hg, with an average of 70 mm. Hg. This rise is, of course, smaller than the maximum obtainable from similar doses in the anesthetized animal (16). Adrenalectomized dogs on maintenance extract show the same order of pressure response.

The data given in figure 1 show that there was no reduction in the capacity of the vasoconstrictor apparatus to respond fully to large doses of renin, despite the falling blood pressure, until rather late in adrenal

insufficiency. Even when the pressure had fallen to 50 mm. Hg or below, the maximum pressor response to these large doses was never entirely lost.

II. *Low renin dosage* (fig. 2). The overdosages of renin obviously measured only the maximum power of constriction, and afforded no direct index to possible changes in sensitivity. This index was obtained by the use of small, clearly sub-maximal doses of renin, given four adrenalectomized dogs in various stages of insufficiency. Figure 2 shows that there was an immediate reduction in response when cortical extract was
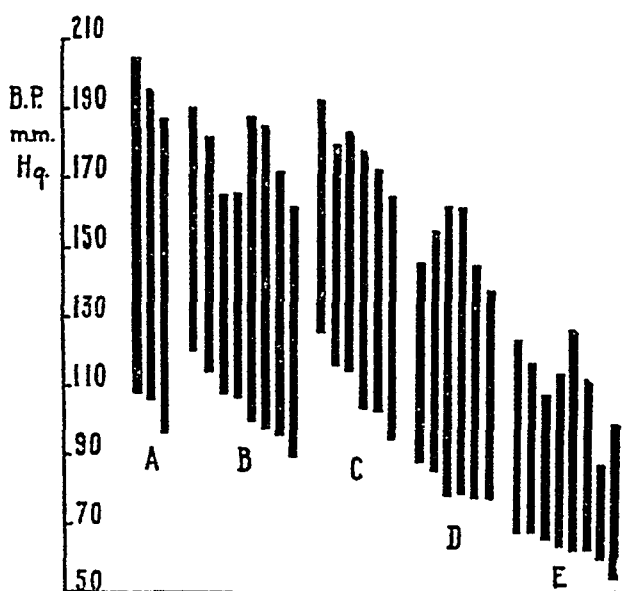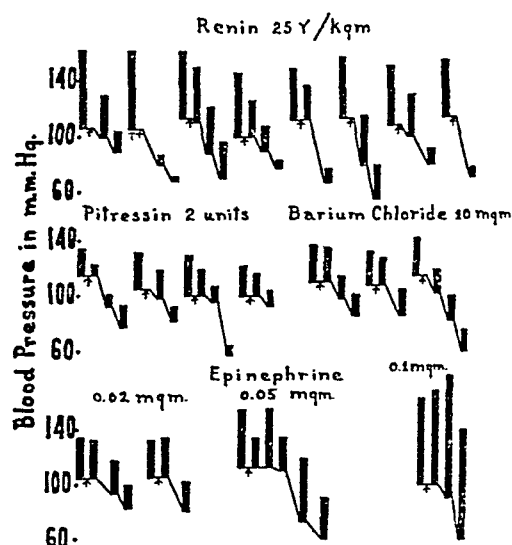
Fig. 1. Blood pressure responses of adrenalectomized dogs in various stages of adrenal insufficiency to overdosages of renin.

A, unilaterally adrenalectomized, average rise 72 mm. Hg; *B*, bilaterally adrenalectomized, maintenance extract, average rise 72 mm. Hg; *C*, extract withdrawn, no symptoms, average rise 69 mm. Hg; *D*, mild insufficiency, average rise 70 mm. Hg; *E*, moderate to severe insufficiency, average rise 48 mm. Hg.

Fig. 2. Blood pressure responses of adrenalectomized dogs in various stages of adrenal insufficiency to low doses of renin.[2]

↑ Cortical extract withdrawn.

[2] Twenty-five gamma per kilogram body weight yielded an average rise of 35 mm. Hg on assay (15).

withdrawn, even while the arterial pressure and blood electrolyte concentrations were still normal. As symptoms of adrenal insufficiency appeared, the responses to renin became progressively less. When the pressure had fallen to 50 mm. Hg, the average rise was only 27 per cent of the normal.

This decline in sensitivity to small doses of renin was strikingly confirmed by a second series of animals. Four adrenalectomized dogs on minimal maintenance doses of cortical extract showed a distinct tendency toward a response lower than that given by control intact animals (table

2). The injection of large amounts of cortical extract corrected this deficiency. The four dogs were then placed on daily doses of 0.5 mgm. desoxycorticosterone acetate[3] for a period of 14 days. The pressor response was equal to that of the intact animals (table 2).

We have not observed, as did Friedman and co-workers (13), a full response to either large or small doses of renin in an animal with blood pressure at shock levels. Otherwise, our results with the low renin dosage agree with those of these workers and those of Williams and co-workers (12). Since the full response is reduced even on barely maintenance levels of cortical extract, and shows a rapid diminution when extract is withdrawn, it is not surprising that the response is often subnormal in animals receiving only salt therapy (12–13).

TABLE 2

The blood pressure responses to small doses of renin* of adrenalectomized dogs on low doses of cortical extract and on maintenance doses of desoxycorticosterone acetate

INTACT CONTROLS				ADRENALECTOMIZED						
					Cortical extract			Desoxycorticosterone acetate		
Dog	Blood pressure	Peak pressure	Rise in pressure	Dog	Blood pressure	Peak pressure	Rise in pressure	Blood pressure	Peak pressure	Rise in pressure
	mm. Hg	mm. Hg	mm. Hg		mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg
1	114	150	36	5	114	142	28	122	154	32
2	104	140	36	6	96	122	26	114	153	39
3	116	153	37	7	124	149	25	114	150	36
4	116	145	29	8	92	120	28	128	152	24
Ave.	113	147	34		106	133	27	120	152	32

* One-tenth milligram per kilogram body weight of an extract yielding an average rise of 34 mm. Hg on assay (15).

It has been reported that the loss in pressor response to renin is not true for other pressor drugs, e.g., tyramine, epinephrine and amphetamine (13). Armstrong and co-workers (18) confirmed Elliott (17) that epinephrine in large doses will give full pressor response in terminal adrenal insufficiency, with the blood pressure at shock levels. The results given in figure 2 lend further confirmation, for there was no reduction in response to a dose of 0.1 mgm. epinephrine. Some variation in response to a dose of 0.05 mgm. was observed, with apparently a tendency for the response to diminish as the blood pressure declined. However, evidence of a lessening of response with 0.02 mgm. was seen. We do not know whether the decreased circulation time present in adrenal insufficiency contributed in

[3] We are indebted to the Ciba Pharmaceutical Products, Inc., for generous supplies of the desoxycorticosterone acetate (Percorten) used in these experiments.

any way to this diminution in pressor response to small doses of epinephrine.

The pressor response to splanchnic stimulation, barium chloride, and pitressin are said to be lost in terminal insufficiency (17–18). If this reduction appears early in insufficiency, it would perhaps be comparable to that seen with small doses of renin. Figure 2 shows that the response to pitressin, like that to renin, is often reduced before the arterial pressure has fallen. The sensitivity to barium chloride is lost more slowly, but it is certainly reduced before the arterial pressure has fallen to shock levels. Consistent results with both these drugs were difficult to obtain in the unanesthetized animal, since secondary reactions such as cardiac slowing, vomiting, etc., often were present. No secondary reactions were seen with either large or small doses of renin.

DISCUSSION

The sensitivity to small doses of renin is reduced in the adrenalectomized dog after cortical extract is withdrawn even while the body weight, appetite, blood pressure and level of blood constituents are still entirely normal. In fact, the sensitivity may be partially reduced when merely maintenance doses of cortical extract are given. To a lesser degree, the sensitivity to pitressin is also often reduced. The writers have repeatedly shown that the adrenalectomized dog on maintenance doses of cortical extract shows far less resistance to shock inducing procedures than either the intact animal or the adrenalectomized dog receiving large priming doses of extract. Both lines of evidence seemingly indicate that the vascular peripheral apparatus shows impairment very shortly after the withdrawal of cortical extract, and before detectable symptoms of adrenal insufficiency have appeared. Desoxycorticosterone acetate will seemingly maintain this ability to respond to pressor agents as effectively as will cortical extract, and it will adequately protect the adrenalectomized dog against most types of shock inducing procedures (21).

When definite symptoms of adrenal insufficiency have become manifest, the ability to respond to small doses of renin is but a fraction of the normal. Likewise the pressor response to pitressin, barium chloride, and probably to small doses of epinephrine has been reduced. The underlying factor in this loss in sensitivity of the vascular periphery of the adrenalectomized dog not receiving cortical extract cannot be defined at the present time. It is not simply an absolute loss in power of arteriolar constriction, for there is usually little consistent reduction in response to large doses of epinephrine, even when the animal is moribund. Large doses of renin elicit full pressor response until relatively late in adrenal insufficiency. Although knowledge of the action of renin is incomplete, it is presumably not sympatho-mimetic (19–20). The contrasting failure of pitressin and

barium chloride to stimulate the smooth musculature of the arterioles in terminal adrenal insufficiency (17–18) could conceivably be a reflection of inadequate dosage.

It would seem not entirely valid to assume that the lessening of the pressor response to renin by the adrenalectomized animal not receiving extract indicates a specific interrelationship between renin and the adrenal cortex, any more than the loss in sensitivity to pitressin and barium chloride is indication of a specific relationship. The decreased ability to respond to pressor drugs, including renin, seemingly reflects the dependence of the functional integrity of some part of the vascular peripheral apparatus upon the presence of hormone or hormones of the adrenal cortex. Our experiments indicate that this hormone may be desoxycorticosterone acetate.

SUMMARY

1. The maximal power of vasoconstriction of the adrenalectomized dog, as measured by the response to overdosages of renin, is not reduced until the arterial pressure has fallen to shock levels.

2. The pressor response to small doses of renin is reduced shortly after cortical extract is withdrawn, and before definite changes in blood chemistry or blood pressure have appeared. In severe insufficiency the response has been largely lost.

3. A partial loss in sensitivity to pitressin and barium chloride, and perhaps to small doses of epinephrine, is also present in early insufficiency. There was no consistent reduction to large doses of epinephrine.

4. The loss in sensitivity to small doses of renin does not necessarily indicate a specific interrelationship between the adrenal cortex and renin, but rather reflects a loss of functional integrity of some part of the peripheral vascular apparatus.

5. Adrenalectomized dogs maintained on the synthetic steroid desoxycorticosterone acetate gave full pressor responses to renin.

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THE CALORIE INTAKE AND WEIGHT BALANCE OF HYPER-THYROID DOGS IN RELATION TO VITAMIN B_1 AND YEAST[1]

VICTOR A. DRILL

From the Section of Physiology, Biological Laboratory, Princeton University, Princeton, N. J.

It has been shown by many investigators that there is a definite relationship between the food intake of an animal and the requirement for undifferentiated vitamin B. In 1927 Cowgill and Klotz showed that the vitamin B requirement of various species of animals could be approximated by the expression (3):

$$\frac{\text{Vitamins}}{\text{Weight} \times \text{Calories}} = K$$

A similar relationship was also shown by Plimmer, Rosedale and Raymond (15).

This equation was tested experimentally by Cowgill et al. (5) by increasing the voluntary food intake of the experimental animals by vigorous exercise, and they found that the vitamin B requirement was increased. This relationship was also tested by increasing the metabolism by the feeding of thyroid gland (4, 10). For example, Himwich et al. (10) found that anorexia was produced in normal dogs, with a yeast free diet, in an average of 34 days, whereas thyroid fed dogs on the same diet showed a loss of appetite in 17 days. During this time the thyroid fed dogs ate twice as many calories as the control dogs. This clearly shows that the amount of vitamin B required by an animal increases in proportion to the amount of food metabolized.

Sure and Buchanan (17) have shown that crystalline vitamin B_1 will partially protect thyroid fed rats against a loss of weight. Drill and Sherwood (7) demonstrated that thyroid fed rats which had lost weight, would regain their lost weight when both vitamin B_1 and yeast were administered, even while the thyroid feeding was continued. Vitamin B_1 supplements alone would stop the loss of weight, but the thyroid fed rats

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did not gain weight until the other B vitamins were administered.   In this paper the food intake and weight balance of thyroid fed dogs has been studied in relation to the yeast and vitamin B_1 content of the diet, the thyroid gland being fed for a long period of time (60–90 days).

METHODS.   The dogs used were full-grown males weighing between 9 and 17 kgm.   They were fed a stock diet of Purina checkers ad libitum before the experiment was started.   Two weeks before the thyroid feeding was begun the dogs were placed on a modified form of Cowgill's casein diet no. III (1), which was then continued throughout the experiment.   In the modified diet 21 per cent of lard was used and the butter was replaced by 4 per cent of cod liver oil (U.S.P.), so that a known amount of vitamins A and D was added to the diet.   The dogs were allowed to eat as much as they wanted for a three hour period each day.   Water was constantly supplied.   Each dog also received a daily supplement of yeast no. 17800

TABLE 1

The initial weight, surface area and supplements fed to each dog

DOG NUMBER	THYROID GLAND FED PER KGM. OF BODY WEIGHT	GRAMS OF YEAST FED PER DAY	INITIAL WEIGHT	SURFACE AREA
			grams	*sq. m.*
1	None	2.0	10,720	0.518
2	None	3.1	16,220	0.674
3	0.4	2.2	11,975	0.575
4	0.4	3.2	16,810	0.730
5	0.4	2.7	14,290	0.629
6	0.4	2.6	13,840	0.651
7	0.6	2.6	13,610	0.676
8	0.6	1.7	8,900	0.493

in the proportion of 2 International Units of vitamin B_1 per pound of body weight.   The yeast used was a dried baker's yeast containing 23 I.U. of vitamin B_1 per gram and 20 Sherman-Bourquin units of vitamin G (flavin) per gram.   This makes the diet normal in all respects and supplies a constant amount of the B vitamins.   The thyroid gland fed was Lilly's desiccated thyroid gland (no. 957625) containing 0.213 per cent iodine. The dogs received 0.4 or 0.6 gram of thyroid per kilogram of body weight (table 1).[2]

The surface area of the dogs was calculated, using the formula of Cowgill and Drabkin (2).   The surface area of the dogs at the start of the experiment is listed in table 1.   If the animals' weight changed to any great extent during the experiment the surface area was recalculated.

RESULTS.   The food intake was calculated as calories consumed per square meter of body surface per hour for each dog.   The two control dogs in this experiment, receiving the modified Cowgill diet and yeast supplement, ate an average of 58.2 calories per square meter per hour.

When thyroid feeding was started a marked increase in appetite was obtained, reaching a peak between the third and fourth weeks (fig. 1). At this time the hyperthyroid dogs were eating an average of 97 calories per square meter per hour, nearly twice that of the controls.   As the thyroid feeding was continued, however, the food intake began to decline.   This drop in food intake is similar to that reported for thyroid fed rats (7),
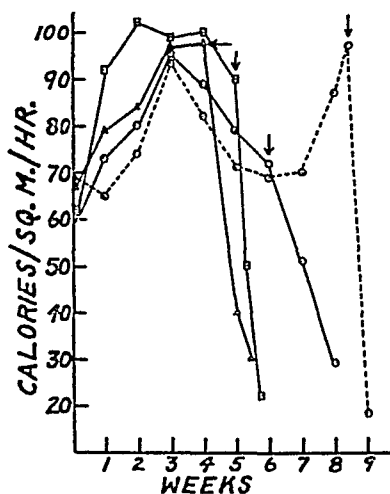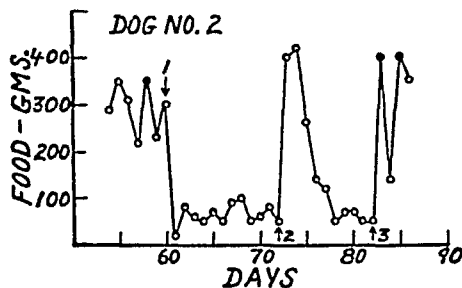
Fig. 1   Fig. 2

Fig. 1. The calorie intake per square meter of body surface per hour of the hyperthyroid dogs.   Each point is an average value of the calorie intake for the number of days following the preceding point on the graph.   Note the marked increase in calorie intake between the second and fourth weeks of thyroid feeding.   The arrow indicates the removal of yeast from the diet, which is followed by the marked and sudden drop in appetite.

Fig. 2. The effect of vitamin B_1 on the food intake of a hyperthyroid dog. 1. Yeast removed from the diet. 2. An injection of 2 mgm. of crystalline vitamin B_1. 3. A second injection of 2 mgm. of crystalline vitamin B_1.

and is indicative of diminished body stores of vitamin B_1.   It has been reported by Drill (6) and confirmed by Peters and Rossiter (14) that thyroid feeding decreases the amount of vitamin B_1 in rat tissues.

The yeast was then removed from the diet of the hyperthyroid dogs and every dog except no. 6 showed an immediate drop in appetite to a very low level (fig. 1).   Some of these dogs even showed a complete anorexia within a few days.   Dog 6 had gained weight after the experiment had started, and was thus receiving less than 0.4 gram of thyroid gland per kilogram of body weight.   Consequently he had not been depleted of his vitamin stores to any great extent, and his loss of appetite was therefore slower

after the yeast was removed from the diet.   Dog 8, receiving 0.6 gram
of thyroid gland per kilogram of body weight, spontaneously showed
anorexia before the yeast was removed from the diet.   Figure 1 clearly
shows that the body stores of the B vitamins were depleted, for if the body
stores were normal it would have taken 17 days to produce anorexia when
the yeast was removed from the diet of the thyroid fed dogs (11).

Weight changes.   The two control dogs, fed the modified Cowgill diet
plus a daily supplement of yeast, made a slight gain in weight.   None of
the dogs fed thyroid gland, except no. 8, lost any weight until the yeast
was removed from the diet.   Dog 8, as mentioned before, was beginning
to show signs of anorexia and had therefore lost some weight.   As soon as
the yeast was removed from the diet of the other hyperthyroid dogs an
immediate loss of weight was obtained.   The loss of weight always followed
the loss of appetite and never preceded it.   Dog 6, as mentioned above,
showed a slower loss of appetite.   The drop in weight of this dog was also
correspondingly slower.   Thus the change in the food intake, and second-
arily the weight of the animal, are not primary effects of thyroid feeding,
but are secondary effects due to a deficiency of the B vitamins.

Vitamin B₁ injections.   The thyroid fed dogs were then maintained on
the modified Cowgill diet for 10 to 15 days, without yeast in the diet.
During this period they lost from 0.75 to 1.5 kgm. in weight.   Each dog
was then injected subcutaneously with 2 mgm. of crystalline vitamin B_1.[3]
Within 24 to 48 hours the food intake was stimulated and rose high above
normal to the hyperthyroid level (fig. 2).   The dog in figure 2 is repre-
sentative of the other hyperthyroid dogs.   The control dogs ate approxi-
mately 187 grams of food per day.   Dog 2 ate an average of 300 grams of
food per day until the yeast was removed from his diet on the 60th day
of the experiment, as shown in figure 2.   The food intake then dropped
below normal and remained low until vitamin B_1 was injected on the 72nd
day.   The food intake then remained high until the vitamin B_1 was metab-
olized and/or excreted.   'On the 82nd day a second injection of 2 mgm. of
vitamin B_1 was given and the food intake again increased above normal
to the hyperthyroid level.   This clearly shows that the high food intake
of the hyperthyroid dog is dependent on a normal supply of vitamin B_1,
and that when the vitamin B_1 drops below the animals' requirements a
rapid drop in appetite ensues, which in turn is followed by a loss of weight.

DISCUSSION.   Kunde (11) and McDonald et al. (12) have made various
studies on hyperthyroid dogs.   They noticed that some of their dogs
would gain weight, some would lose weight, and that others would show
no change in weight.   Kunde stated that her results indicated that neither

[3] The crystalline vitamin B_1 was kindly supplied by Dr. R. T. Major of Merck
and Co. as Betabion (Thiamin chloride).

the amount of thyroid fed nor the height of the basal metabolic rate of the dogs determined the percentage loss of weight. Their dogs were fed commercial diets, of which the vitamin potency was not known. In such diets the vitamin intake varies directly with the food intake. Their results are undoubtedly due to a variable food intake with a consequent different vitamin intake. Earlier work on rats, and the present work on dogs, shows the importance of feeding synthetic diets containing a known amount of yeast in all cases where hyperthyroidism is being studied.

In this experiment each dog received a daily supplement of yeast, in proportion to body weight, which is sufficient for normal maintenance. However, if the requirement for the B vitamins is increased the dogs will soon show symptoms of a vitamin B_1 deficiency. Thus the feeding of thyroid gland gave an initial increase in appetite, but as the dietary yeast remained constant the calorie intake soon began to drop. It has already been shown that thyroid feeding will decrease the vitamin B_1 stores of thyroid fed rats (6, 14). The removal of the yeast from the diet at this point causes a rapid decrease in appetite to subnormal levels, some animals showing complete anorexia. With the loss of appetite a decrease in body weight occurs. After the yeast was removed for a few days, and the appetite and weight had fallen, the animal was injected with 2 mgm. of crystalline vitamin B_1 and within 48 hours the appetite had risen to the previous hyperthyroid level. An increase in weight was also obtained. After a few days the vitamin B_1 was excreted and the appetite again fell to subnormal levels, from which it could be raised by injecting vitamin B_1 again.

The experimental work suggests that the continued anorexia and loss of weight in Graves' disease, after iodine therapy, is due to a deficiency of vitamin B_1. Harris and Leong (9) studied the excretion of vitamin B_1 in the urine of humans and concluded that the amount of this vitamin stored in the body was quite small, and that the maintenance of the vitamin stores was dependent on an adequate intake in the diet. Schneider and Burger (16), using the method of Jansen, reported that control patients excreted 80 to 100 gamma of vitamin B_1 per day in the urine, whereas patients with Graves' disease excreted an average 224.8 gamma of vitamin B_1 per day. After operation the vitamin B_1 excretion fell to a normal average of 79.9 gamma per day.

Means et al. (13) have mentioned that yeast increased the appetite and weight of a small series of thyrotoxic patients. Frazier and Ravdin (8) studied the effect of vitamin B_1 on the preoperative preparation of the hyperthyroid patient. They found that 72 per cent of the vitamin treated group gained weight, whereas only 28.5 per cent of the control group gained weight. The vitamin treated group showed a greater increase in appetite than the control group. This is the improvement that would be expected

if some degree of vitamin B_1 deficiency existed. The greatest improvement in the vitamin treated group was observed in the more toxic patients, which is the group in which a vitamin deficiency is more likely to develop.

The evidence presented shows that a deficiency of vitamin B_1 can occur in hyperthyroid dogs *which contain a minimal but normal amount of yeast in the diet.* This deficiency occurs before the weight drops. When the appetite had begun to decline, the removal of the yeast from the diet caused anorexia within a few days, whereas in an animal with normal body stores of vitamin B_1 the removal of the dietary yeast resulted in anorexia in 34 days. In human patients with moderate or severe hyperthyroidism the vitamin B_1 requirement may also be increased sufficiently to produce a loss of weight and anorexia.

SUMMARY AND CONCLUSIONS

1. The food intake of 6 dogs, receiving a normal diet containing yeast, increased to nearly twice the normal level when thyroid feeding was started. After 3 to 4 weeks of thyroid feeding the food intake began to decline, indicating depletion of the body stores of the B vitamins.

2. When the yeast was removed from the diet of the hyperthyroid dogs the appetites rapidly fell below normal, complete anorexia being produced in some cases. The subcutaneous injection of 2 mgm. of crystalline vitamin B_1 restored the appetite to the previous hyperthyroid level within 24 to 48 hours.

3. The decrease in appetite of the thyroid fed dogs is due to a deficiency of vitamin B_1. Anorexia is not a primary effect of hyperthyroidism but is a secondary symptom due to a deficiency of vitamin B_1.

4. The weight of the hyperthyroid animals was maintained as long as no deficiency of the B vitamins occurred. As soon as the yeast was removed from the diet a drop in food intake took place which was followed by a secondary loss of weight.

5. The previous variability of the weight of hyperthyroid dogs reported by other authors is probably due to the difference in the intake of the B vitamins in the diet of the animals.

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THE EFFECT OF THIAMIN ON THE INTESTINE OF THE B₁-DEFICIENT RAT

MACDONALD DICK AND J. ROY HEGE, JR.

From the Department of Physiology and Pharmacology, Duke Hospital, Durham,
North Carolina

It has frequently been observed that crude vitamin B products increase intestinal motility of deficient animals. Observations made after the identification of thiamin as the anti-neuritic principle suggest that vitamin B_1 is the factor responsible for the effect. Indeed, Agid, Beauvallet and Minz (1) have shown that the sensitivity to acetylcholine of the isolated rat intestine is increased in the presence of thiamin and this was found to be true for the intestine of the B-deficient pigeon (2). From these considerations it appears that it might be possible to demonstrate an effect of thiamin on the intestines of normal as compared with B_1-deficient animals and this investigation was undertaken to determine whether an immediately demonstrable effect could be established.

EXPERIMENTAL. The apparatus used was essentially that of Polansky (3). Slight changes in our apparatus made easier the substitution of successive strips of ileum without changing the physical principles involved. An excellent account of these is given by Polansky. With the apparatus any desired liquid can be passed through the lumen of a strip of intestine at controlled rates and pressures. The recording of longitudinal and circular contractions and the maintenance of a constant temperature are provided for.

The rats, all approximately 3 months old, were fed the following diet until a definite weight loss was observed: casein 20; sugar 52; powdered agar 3; salts 5; autoclaved yeast 20; two drops of cod liver oil per day. The controls received in addition 1 mgm. of thiamin per day. All animals were therefore fed on a diet of similar physical properties to avoid changes in motility caused by such a difference in the food eaten.

Since preliminary observations showed that urethane injected intraperitoneally into the rat had no effect on the motility of the ileum as already shown by Alvarez and Hosoi (4), some of the animals were anesthetized with urethane while others were decapitated. Anesthetization with urethane permits the use of many segments of intestine from the same animal. After the rat, which had previously been starved for 24 hours, was

killed or anesthetized, the abdominal cavity was opened, a strip of intestine 5 to 7 cm. long removed from the distal end of the ileum and the abdominal cavity covered until another strip was needed.

The segment of intestine was attached to the apparatus and warm (37°C.) Ringer's solution passed both outside the intestine and through the lumen while the contractions were recorded continuously. If desired, then, the fluid passing through the lumen was changed to thiamin chloride $(1:10^6)$ in Ringer's. It is well to emphasize that the pressure within the strip of intestine was unchanged when the perfusing fluid was changed; if this were not so, changes in motility might have been the result of factors other than the addition of thiamin.

For the intestine of normal animals the preliminary experiments indicated that the addition of thiamin to the perfusing fluid had no effect demonstrable by this method, which was now applied to the B₁-deficient
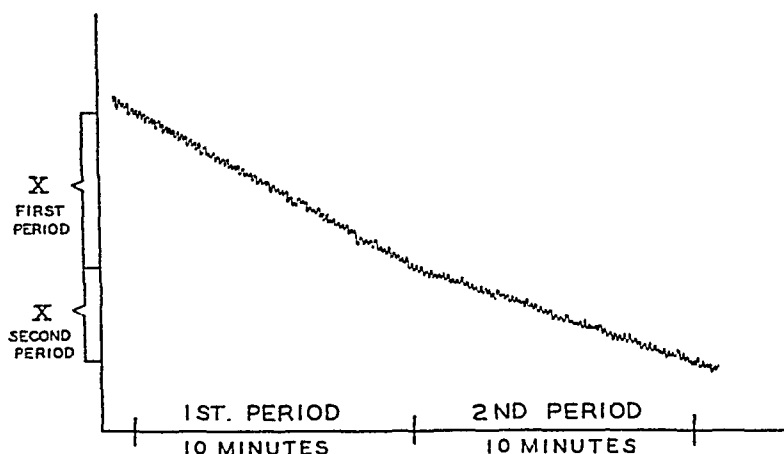
Fig. 1. Showing measurement of tonus loss

rats. Records of activity of 27 strips of intestines from deficient animals were taken with the addition of thiamin to the perfusing fluid after an initial period of perfusion with Ringer's. Likewise records of 15 strips were made without the addition of thiamin but perfused with Ringer's solution throughout.

The amplitudes of contraction were measured and recorded for successive ten minute periods before and after the addition of thiamin and for an equal interval without thiamin. From the beginning of the experiment the strip of intestine gradually lost tonus as indicated by a steady fall in the pointer registering contractions. The vertical distance through which the lever fell in unit time is a measure of the rate of loss of tonus and is so recorded in the table. Figure 1 illustrates the type of curve and the readings recorded.

RESULTS. The table (table 1) shows that for deficient animals the rate of loss of tonus is retarded after the addition of thiamin while the ampli-

TABLE 1

ANIMALS ON ADEQUATE DIET

Rate of loss of tonus, mm./10 min.						Amplitude of contraction, mm.					
Thiamin added at end of 1st period			No thiamin added			Thiamin added at end of 1st period			No thiamin added		
Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.
1.2	0.7	−0.5	0.6	0.6	0.0	0.40	0.40	0.00	0.30	0.20	−0.10
1.3	1.0	−0.3	0.5	0.3	−0.2	0.30	0.10	−0.20	0.90	0.90	0.00
1.1	1.0	−0.1	0.5	0.3	−0.2	1.20	0.90	−0.30	1.70	1.80	0.10
1.4	1.5	+0.1	1.2	0.5	−0.7	1.10	1.10	0.00	0.50	0.50	0.00
1.3	1.2	−0.1	0.4	0.6	+0.2	0.90	0.90	0.00	0.30	0.20	−0.10
0.8	0.5	−0.3	0.1	0.1	0.0	0.70	0.60	−0.10	0.10	0.10	0.00
1.2	1.0	−0.2	1.0	0.7	−0.3	0.10	0.03	−0.07	0.20	0.20	0.00
1.0	1.0	0.0	1.2	0.9	−0.3	1.20	1.20	0.00	0.20	0.20	0.00
1.0	0.7	−0.3	1.2	0.6	−0.4	1.10	1.10	0.00	1.50	1.50	0.00
1.1	1.1	0.0				0.80	0.80	0.0			

Rate of loss — Thiamin added: $\bar{x}_1 = -0.17$, $\sigma^2 = 0.03344$
Rate of loss — No thiamin added: $\bar{x}_2 = 0.2\bar{1}$, $\sigma^2 = 0.06861$
$t_{x_1 x_2} = 0.4$ (P > .99)

Amplitude — Thiamin added: $\bar{y}_1 = -0.067$, $\sigma^2 = 0.011$
Amplitude — No thiamin added: $\bar{y}_2 = 0.02\bar{2}$, $\sigma^2 = 0.0030$
$t_{y_1 y_2} = 1.3$ (P > .9)

ANIMALS ON B₁-DEFICIENT DIET

Rate of loss of tonus, mm./10 min.						Amplitude of contraction, mm.					
Thiamin added at end of 1st period			No thiamin added			Thiamin added at end of 1st period			No thiamin added		
Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.
1.7	0.5	−1.2	0.1	1.8	+1.7	0.20	0.30	0.10	0.27	0.10	−0.17
2.7	1.2	−1.5	0.3	1.6	+1.3	0.00	0.40	0.40	0.30	0.50	0.20
1.8	0.0	−1.8	1.6	1.3	−0.3	0.10	0.60	0.50	0.80	0.40	−0.40
1.6	0.9	−0.7	4.0	1.8	−2.2	0.05	0.05	0.00	0.40	0.20	−0.20
0.6	0.2	−0.4	2.0	1.6	−0.4	0.00	0.30	0.30	0.40	0.40	0.00
1.6	0.5	−1.1	0.2	0.8	+0.6	0.50	0.53	0.03	0.15	0.20	0.05
1.8	0.5	−1.3	0.8	0.5	−0.3	0.15	0.55	0.45	0.20	0.06	−0.14
3.8	1.0	−2.8	3.0	3.0	0.0	0.20	0.30	0.10	0.06	0.09	0.03
1.6	0.4	−1.2	0.9	1.7	+0.8	0.09	0.16	0.07	0.05	0.10	0.05
0.5	0.2	−0.3	1.7	0.9	−0.8	0.10	0.50	0.40	0.30	0.20	−0.10
3.0	1.5	−1.5	0.9	1.5	+0.6	0.10	0.15	0.05	0.60	0.70	0.10
1.0	0.4	−0.6	0.5	2.0	+1.5	0.70	0.70	0.00	0.06	0.03	−0.03
0.9	0.5	−0.4	3.9	1.7	−2.2	0.03	0.03	0.00	0.01	0.06	0.05
1.5	0.1	−1.4	6.0	3.5	−2.5	0.40	0.40	0.00	0.10	0.05	−0.05
2.0	0.7	−1.3	1.3	1.3	0.0	0.05	0.10	0.05	0.40	0.20	−0.20
1.6	1.3	+0.3				0.04	0.04	0.00			
2.5	1.9	−0.6				0.04	0.10	0.00			
2.4	0.4	−2.0				0.10	0.20	0.00			
0.8	0.3	−0.5				0.25	0.10	−0.15			
2.7	1.3	−1.4				0.05	0.20	0.10			
2.7	0.8	−1.9				0.05	0.30	0.25			
1.4	0.7	−0.7				0.24	0.24	0.00			
1.2	0.5	−0.7				0.04	0.08	0.04			
2.6	0.9	−1.7				0.02	0.05	0.03			
1.2	0.9	−0.3				0.14	0.21	0.07			
1.3	0.6	−0.7				0.00	0.17	0.17			
						0.04	0.10	0.06			

Rate of loss — Thiamin added: $\bar{x}_1 = -1.05$, $\sigma^2 = 0.44423$
Rate of loss — No thiamin added: $\bar{x}_2 = -0.145$, $\sigma^2 = 1.76981$
$t_{x_1 x_2} = 2.85$ (P < .01)

Amplitude — Thiamin added: $\bar{y}_1 = 0.117$, $\sigma^2 = 0.02609$
Amplitude — No thiamin added: $\bar{y}_2 = -0.054$, $\sigma^2 = 0.02261$
$t_{y_1 y_2} = 3.28$ (P < .01)

tude of contraction is increased. The variation among the normal as well as the deficient animals is considerable but the differences in the motilities under the two conditions are apparent. These are confirmed by statistical analysis where the t-values show significant differences for the deficient but not for the normal animals.

The results agree with the work of Abderhalden and Abderhalden (5) recently extended by Briem (6) which showed that thiamin increases the action of acetylcholine. This in turn is explained by the work of Glick and Antopol (7) who showed that the hydrolysis of acetylcholine was slowed down in the presence of thiamin because of the greater affinity of the esterase for thiamin as compared with acetylcholine.

Thus thiamin in concentrations close to those that might be found in the body does act at once to increase the activity of the intestines of vitamin B₁-deficient rats. While this does not necessarily assert that the total effect of thiamin in improving bowel action is only directly upon the gut, it is clear that a direct effect is important. The integration of many such small increases as observed here *in vitro* applied along the whole length of the gut could conceivably become very effective.

<center>SUMMARY</center>

Thiamin chloride perfused through the isolated intestine of a normal rat has no effect within the limits of the method of observation used in these experiments. On the other hand, the peristalsis of the intestines of a B₁-deficient rat is increased and the rate of tonus loss retarded within ten minutes after the addition of thiamin.

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CEREBRAL BLOOD FLOW AND BRAIN METABOLISM DURING INSULIN HYPOGLYCEMIA[1]

H. E. HIMWICH, K. M. BOWMAN, C. DALY, J. F. FAZEKAS, J. WORTIS AND W. GOLDFARB

From the Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, New York and Psychiatric Division, Bellevue Hospital, New York City

Sakel's treatment for schizophrenia has afforded an opportunity to study brain metabolism of the human during hypoglycemia. A decrease in the cerebral arterio-venous oxygen difference has been reported (1) (2). This was interpreted to indicate a decrease of brain metabolism because Loman and Myerson (3) have demonstrated a fall of blood flow through the human brain during insulin hypoglycemia, and Leibel and Hall (4) found no significant change in the cerebral blood flow of rabbits subjected to insulin coma unless convulsions occurred. Abramson et al. (5), however, observed an increased peripheral blood flow through the extremities during the insulin treatment. Though their data yield no evidence on cerebral blood flow, these workers nevertheless suggested that the smaller arterio-venous oxygen difference during insulin hypoglycemia was caused by a faster blood flow. It is true that conclusive information regarding changes of brain metabolism could be gained only by determining simultaneously both cerebral blood flow and arterio-venous oxygen difference. For this reason arterio-venous oxygen differences and cerebral blood flow were determined in patients with schizophrenia. The effects of various substrates on brain metabolism during hypoglycemia were also observed.

METHOD. Methods for the collection and analysis of the blood samples have been previously described (2). In addition pyruvic acid (6) and bisulfite binding substances (7) were determined.[2] The rate of blood flow in the internal jugular vein was estimated by a modification of the Gibbs thermostromuhr. This instrument proved to be at least as sensitive as the original and has an error of ± 10 per cent for a single reading. For that reason repeated readings were made to determine

TABLE 1

Effect of glucose on arteriovenous oxygen differences and cerebral blood flow during insulin hypoglycemia

1	2	3	4	5	6	7
PATIENT AND DATE	TIME	OXYGEN DIFF.	GLUCOSE ART.	PER CENT CHANGE BLOOD FLOW		REMARKS
				Observed	Calculated	
				per cent	*per cent*	
Br 6/21/40	8:10	6.14	83			110 units insulin 8:40
	10:40	4.07	19	−10	+51	
	11:40	2.16	15	−30	+184	
	12:40	2.33	17	−10	+168	
	1:40	1.34	13	0	+358	
C 6/22/40	8:50	6.84	89			425 units insulin 9:15
	10:15	7.53	65			
	11:15	6.95	43	−10	+4	
	12:15	6.25	33	−10	+15	
	1:15	5.46	24	+10	+32	
	2:15	3.72	32	−20	+94	
T 6/24/40	9:30	6.58	101			140 units insulin 10:00; 25 gm. glucose 1:55; 1:58 aroused
	11:50	3.14	35	0	+104	
	1:45	3.55	29	0	+85	
	2:35	6.46	122	−30	+2	
C 7/1/40	9:30	6.69	97			500 units insulin 9:37; 25 gm. glucose 2:39; 2:43 aroused
	1:25	4.59	43	+10	+46	
	2:30	4.17	39	+20	+60	
	3:33	5.70	22		+17	
Br 7/5/40	10:08	6.57	94			125 units insulin 10:16; 25 gm. glucose 1:42
	12:06	0.62	23	−20	+960	
	1:33	2.45	22	−30	+168	
	2:06	6.62		0	−1	
Br 6/27/40	10:54	1.54	34			25 gm. glucose 10:56; 10:59 aroused
	11:15	5.32	117	−20	−246	
B 6/27/40	12:04	3.66	38			25 gm. glucose 12:18; 12:22 aroused
	12:36	6.10	139	−10	−67	
W 6/29/40	11:01	4.16	53			25 gm. glucose 11:16
	11:28	6.08	127	+10	−46	

blood flow at any given moment. Observations on the blood flow were made over periods up to six hours during which time the position of the thermostromuhr needle, in respect to the vein, was not changed. A special

technique was devised which prevented error resulting from the formation of clots on the needle and served to detect any change in tissue blood flow which may have taken place during the experiment.   This method will be described in detail elsewhere.   The patients were studied before the injection of insulin and during coma when glucose, sodium lactate or sodium pyruvate was injected intravenously.

TABLE 2

Effect of insulin hypoglycemia on brain metabolism

1	2	3		4	5
		ARTERIO-VENOUS DIFFERENCE VOLUMES PER CENT			
PATIENT AND DATE	BLOOD FLOW RATIO	Observed		Corrected	BRAIN METABOLISM
					per cent
Br	1.00	6.14		6.14	100
6/21/40	0.90	4.07		3.66	60
	0.70	2.16		1.51	25
	0.90	2.33		2.09	34
	1.00	1.34		1.34	22
C	1.00	7.18		7.18	100
6/22/40	0.90	6.95		6.29	88
	0.90	6.25		5.63	78
	1.10	5.46		6.01	84
	0.80	3.72		2.98	41
T	1.00	6.58		6.58	100
6/24/40	1.00	3.14		3.14	48
	1.00	3.55		3.55	54
	0.70	6.46		4.72	72
C	1.00	6.69		6.69	100
7/1/40	1.10	4.59		5.05	75
	1.20	6.17		5.00	75
	1.00	5.70		5.70	85
Br	1.00	6.57		6.57	100
7/5/40	0.80	0.62		0.50	8
	0.70	2.45		1.72	26
	1.00	6.62		6.62	101

RESULTS.   Table 1 presents a summary of the observations of cerebral blood flow and arterio-venous oxygen difference before and after the injection of insulin as well as those changes caused by the administration of glucose during hypoglycemia.   In 5 cases blood flow and arterio-venous oxygen differences were determined before the injection of insulin as well as throughout the course of the coma and the subsequent administration of

glucose. In 3 other instances observations were begun during coma and continued until after arousal with intravenous glucose. In column 6 are presented the calculated changes in blood flow necessary to cause the changes of the cerebral arterio-venous differences if brain metabolism remained unchanged.[3] Column 5 contains the observed changes in blood flow. In none of these experiments could the low arterio-venous oxygen difference during hypoglycemia be accounted for by any change in blood flow, and in most cases the changes in blood flow were opposite in direction, showing a slow blood flow with a low arterio-venous difference. The average change of 14 observations made during hypoglycemia is −7 per cent.

TABLE 3

Effect of lactate and pyruvate on arterio-venous oxygen differences during insulin hypoglycemia

PATIENT AND DATE	TIME	OXY-GEN DIFF.	GLUCOSE		LACTIC ACID		PYRUVIC ACID		REMARKS
			Art.	Ven.	Art.	Ven.	Art.	B.B.S.	
Br 7/8/40	12:30	1.63	17.0	12	13.0	10			20 gm. r-sodium lactate 12:35
	12:44	1.93	17.0	14	40.0	43			
T 7/8/40	11:40	4.64	20.0	10	16.0	12			20 gm. r-sodium lactate 11:44
	11:56	3.58	19.0	8	24.0	23			
W 7/9/40	11:25	2.65	19.0	15	10.0	8			20 gm. r-sodium lactate 11:32
	11:40	2.58	13.0	13	25.0	27			
Br 7/10/40	11:30	2.13	19.0	24	14.0		1.50		7.5 gm. sodium pyruvate 11:37
	11:50	2.61		25	31.0		2.86	9.35	
L 7/10/40	12:35	3.14	12.0	7	12.0		1.32	5.96	7.5 gm. sodium pyruvate 12:38
	12:50	2.79	12.0	12	28.0		2.94	8.81	
T 7/11/40	11:27	4.79	28.0	25	9.0		1.37	3.26	10 gm. sodium pyruvate 11:30
	11:40	4.08	19.0	16	18.0		4.13	8.10	

In table 2 are (column 2) the ratios between the blood flow during hypoglycemia and before the injection of insulin; (column 3), the observed arterio-venous oxygen difference; (column 4), the arterio-venous oxygen difference corrected for blood flow (column 2 × column 3) and column 5 contains per cent brain metabolism (column 4 ÷ control). The results of the injection of sodium lactate and sodium pyruvate are also tabulated (table 3). In these experiments neither the arterio-venous oxygen difference nor the cerebral blood flow changed markedly.

[3] $\dfrac{\text{Control} - \text{observed value}}{\text{observed value}}$ = per cent change, e.g., table 1, Br, 6/21/40, $\dfrac{6.14 - 4.07}{4.07}$ = 51 per cent.

DISCUSSION. The interrelationships of the arterio-venous oxygen differences and cerebral blood flow can be brought out in a discussion of the results of a typical example. Patient Br, 6/21/40 (table 1) started with an initial arterio-venous oxygen difference of 6.14 volumes per cent and a blood sugar of 83 mgm. per cent. He then received insulin and his blood sugar fell during a period of four hours to low levels. Meanwhile his arterio-venous oxygen difference decreased gradually to 4.07, 2.16, 2.33, and 1.34 volumes per cent. If these differences were caused by more rapid blood flow, the velocity would have to increase, as seen in the 6th column of table 1, 51, 184, 168, and 358 per cent respectively. However, the observations of blood flow reveal quite the reverse. The blood flow was not faster but slower, −10, −30, −10, and 0 per cent. In none of the present experiments could the smaller arterio-venous oxygen difference during hypoglycemia be accounted for by any change of blood flow and in most cases the changes in blood flow were opposite in direction showing a slow blood flow with a low arterio-venous oxygen difference.[4] By taking into consideration both blood flow and arterio-venous oxygen difference, it is seen that brain metabolism may decrease to approximately $\frac{1}{4}$ of the original value during hypoglycemia (table 2, column 5). The second observation of Br, 7/5/40, is probably too low. In a previous publication (8) we have recorded an average value of 1.77 volumes per cent during deep coma. With an average decrease of cerebral blood flow of 7 per cent observed in the present experiments, this indicates that brain metabolism during hypoglycemia may be reduced to 24 per cent of the original.

The effects of the injection of glucose are seen in Br, 6/27/40. The arterio-venous oxygen difference rose from 1.54 to 5.32 volumes percent, while blood sugar increased from 34 to 117 mgm. per cent following intravenous administration of glucose. Such an increase might be caused either by a greater cerebral metabolism or a slower cerebral blood flow. In this observation, blood flow would have to decrease 246 per cent to cause such a change in the arterio-venous oxygen difference. Actually it decreased only 20 per cent. Thus, the greater arterio-venous oxygen difference after the administration of glucose must be accounted for in the largest part by the augmented metabolism of the brain. This striking increase in cerebral metabolism is reflected in the changed neurological condition of the patient. Within three minutes after receiving 25 grams of glucose intravenously, the patient was aroused and in contact with his environment. Depending on various factors some time is required before

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[4] We have recently been informed by Dr. C. D. Aring, University of Cincinnati College of Medicine, that he has obtained similar results on cerebral blood flow during hypoglycemia though he has used an entirely different method for the determination.

brain metabolism returns to normal.   In T, 6/24/40, and C, 7/1/40, full recovery had not occurred at the time the final observations were made, but in Br, 7/5/40, the brain metabolism was the same as the original.

Because of the definite changes produced by the administration of carbohydrate, Wortis and Goldfarb (9) suggested that hypoglycemia may be employed to determine whether substances other than glucose can support brain metabolism.   Lactic acid and pyruvic acid, which are intermediary products of carbohydrate metabolism, were studied in this manner.   In previous work (10), 20 grams of r-sodium lactate were injected into patients during therapeutic hypoglycemic insulin coma.   The average arterio-venous oxygen difference was 2.71 volumes per cent during coma.   Five to twenty minutes after the injection of lactate the arterio-venous oxygen difference had increased to 4.01 volumes per cent and 25 to 50 minutes afterwards to 4.87 volumes per cent.   This increase in cerebral metabolism was not adequate to arouse the patients from coma.   Similar results were obtained with sodium pyruvate (11) which increased the cerebral arterio-venous oxygen difference from 2.90 to 4.06 volumes per cent.   It is nevertheless possible that in these experiments there might have been a greater increase in cerebral metabolism but a larger arterio-venous oxygen difference was prevented from developing by a more rapid blood flow through the brain.   For that reason experiments were performed in which in addition to determining the arterio-venous oxygen difference before and after the injection of lactate and pyruvate, observations of cerebral blood flow were also made.   The injection of racemic sodium lactate (Br, 7/8/40) produced no significant change either in the arterio-venous oxygen difference, 1.63 and 1.93 volumes per cent, or in the cerebral blood flow despite the fact that lactic acid increased in the arterial blood from 13 to 40 mgm. per cent as may have also occurred in other tissues including the brain.   Probably some lactic was converted to glycogen in the liver and left that organ to reënter the blood stream as glucose (12), but the rate of entry of this hepatic glucose into the blood was not rapid enough to satisfy the tissue demands augmented, as they were, by the injection of insulin.   Blood sugar, therefore, did not increase.   In any case lactic acid did not affect brain metabolism.   Similar results were obtained with pyruvic acid (Br, 7/10/40).   Though the pyruvate content of the blood increased, the arterio-venous oxygen difference and cerebral blood flow remained unchanged within the error of the methods.   Arterial blood sugar was unaltered.   Pyruvate rose from 1.50 mgm. per cent to 2.86 mgm. per cent and a significant amount of pyruvate was changed to lactate which increased from 14 mgm. per cent to 31 mgm. per cent.   The increase of the pyruvate was responsible in part for the somewhat greater rise of bisulfite binding substances.   Thus despite greater concentrations of both pyruvic

and lactic acids, brain metabolism was not stimulated sufficiently to arouse the patient.   Maddock et al. (13) observed that pyruvate was not able to reëstablish brain waves during hypoglycemia in hepatectomized dogs.

These observations are in striking contrast with those obtained with glucose in which the administration of 8 grams or even 4 grams resulted in the stimulation of brain metabolism and the awakening of the patients (14). These results do not mean that lactic and pyruvic acids are not oxidized by the brain but that their oxidation occurs at a rate which is too slow to support cerebral functions.

Lactate and pyruvate are equal to glucose in ability to increase the oxygen uptake of *excised* cerebral tissues.   Apparently a change has occurred in these tissues which renders lactic and pyruvic acids susceptible to more rapid oxidation.   The period of anoxia after the sacrifice of the animal as well as the slicing or mincing of the tissues produces an increase of permeability which may permit lactic and pyruvic acids faster access to the respiratory enzymes.   The slower utilization of lactate and pyruvate during hypoglycemia may be due to the more rapid diffusion of glucose in the brain.   In the present experiments the injected lactate was not absorbed in amounts adequate for demonstration by comparison of its concentrations in arterial and venous blood.   The lactic acid produced in the cell does not encounter such a barrier.   When the level of lactate is elevated considerably above the normal value, the absorption of lactic acid is revealed even in a single circulation through the brain of a normal (15) or diabetic animal (16).   Despite such high concentrations of lactate, patients were not aroused from hypoglycemic coma (10).   The possibility remains that lactic and pyruvic acids may be oxidized more slowly than glucose *in vivo*.   It would then be necessary to seek a second path for glucose metabolism not involving the intermediary stages of lactic and pyruvic acids.   Such a path has been demonstrated by the use of various inhibitors: nicotine (17), hydroxymalonate (18), glyceraldehyde (19), and iodoacetate (20).   These substances interfere with the oxidation or formation of lactic acid without stopping the oxidation of glucose.   An explanation of the present results is at hand if the second path of oxidation is more rapid than the one including lactic and pyruvic acids.   Thus two factors may be operative in facilitating a rate of utilization of glucose more rapid than that of lactic and pyruvic acids.   The brain cells may be more permeable to glucose and once glucose gets into the cell its rate of oxidation may be faster.

SUMMARY AND CONCLUSIONS

The cerebral arterio-venous oxygen difference and cerebral blood flow were determined on patients with schizophrenia during insulin hypoglycemia.   The changes in blood flow during insulin hypoglycemia un-

complicated by convulsions are not of great magnitude and usually exhibit a slight diminution, averaging -7 per cent. Thus, a decrease in the arterio-venous oxygen difference during insulin hypoglycemia is caused by an impaired cerebral metabolism which may be reduced to about $\frac{1}{4}$ of the original value. The injection of glucose during hypoglycemia has no marked effect on the brain blood flow but the arterio-venous oxygen difference increases. This indicates that the cerebral metabolic rate rises though full recovery may not occur immediately. Lactate and pyruvate are not as effective as glucose in restoring brain metabolism. This phenomenon is discussed briefly.

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OBSERVATIONS ON THE RÔLE OF THE THEBESIAN VEINS AND LUMINAL VESSELS IN THE RIGHT VENTRICLE[1]

RICHARD W. ECKSTEIN,[2] JOSEPH T. ROBERTS,[3] DONALD E. GREGG AND JOSEPH T. WEARN

From the Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio

Since Vieussens (1) and Thebesius (2) first demonstrated the existence of minute vessels connecting the coronary vessels with the chambers of the heart, many investigators have studied the anatomy and function of these channels. Wearn and Zschiesche (3) showed that there are two sets of vessels, namely: 1, the Thebesian veins which join the coronary veins with the ventricular cavities, and 2, the luminal vessels which connect the coronary arteries with the ventricular chambers. The question as to whether these vessels conduct blood from the cardiac chambers into the myocardium in the normal heart has not been answered to our satisfaction. Accordingly, experiments were designed to study the direction of blood flow in those vessels which communicate with the right ventricle.

Method A. Dogs were anesthetized with morphine and sodium pentobarbital and their chests were opened under artificial respiration. The hearts were exposed and suspended in a pericardial cradle. The blood was rendered non-coagulable with chlorazol fast pink or pontamine fast pink (0.3 gram per kilo). Ventricular pressures were recorded with large needles connected to Gregg optical pressure manometers (4). The circulations of the right and left hearts were separated with a special cannula, figure 1, inserted into the pulmonary artery. It was arranged so that blood flowed from the pulmonary artery, *PA*, through stopcock *A*, and then into the lungs. About 250 cc. of a 20 per cent aqueous solution of Higgins' waterproof India Ink, or the same volume of a 2 per cent aqueous solution of Berlin Blue was injected into the right ventricle. These substances were injected by gravity at a pressure of about 60 mm. Hg. At the moment the injection was started stopcock *A* was turned one-quarter turn and the mixture of blood and dye from the pulmonary artery, *PA*,

flowed into the rubber bag, *RB*, and displaced an equal volume of oxygenated, heparinized blood from chamber *BL* into the lungs. During the injection continuous right and-left ventricular pressures were recorded. The hearts were stopped with 10 to 15 cc. of 5 per cent KCl injected into the left ventricle. After 10 to 15 progressively smaller beats the hearts came to a complete standstill. Experiments in which fibrillation occurred were not included in the series. Each heart was removed carefully, inspected, fixed in formalin, and studied microscopically.

RESULTS. In six successful experiments, with right ventricular systolic pressure lower than left ventricular systolic-pressure, there was no gross
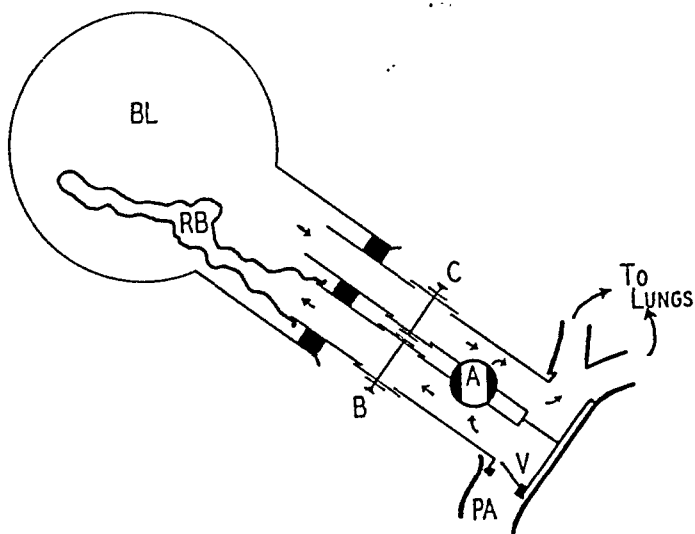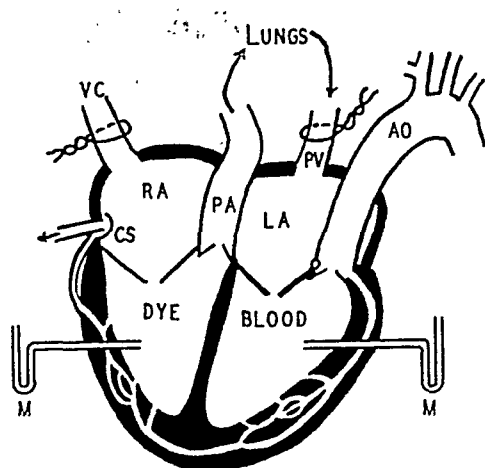
Fig. 1   Fig. 2

Fig. 1. Diagram of special pulmonary cannula inserted into the main pulmonary artery. *A*, stopcock to allow blood to flow directly through cannula. *B* and *C*, clamps on tubes leading to and from chamber *BL* containing a large rubber bag, *RB*, and oxygenated heparinized blood. *V*, one-way rubber flap valve. *PA*, pulmonary artery.

Fig. 2. Diagram of circulation in method B. *PV*, ligature about pulmonary veins. *VC*, ligature about vena cava. *M*, *M*, manometers for measuring ventricular pressures.

injection in any of the hearts. Microscopically, occasionally two to five small vessels on the right side of the intraventricular septum were filled with dye or ink. Rarely there were a few small areas of myocardial capillary injection and more frequently a few larger injected vessels resembling sinusoids or venules. In no case was ink or dye found in large coronary arteries or veins. (See fig. 3.) In one fibrillating heart injection of dye into the right ventricle under pressure of 60 mm. Hg produced a fair amount of capillary injection in both the right and left ventricles.

Method B. Because of the great technical difficulties of the former method and in order to be able to vary the right and left ventricular pres-

sures independently, the following preparation was made: a ligature was placed through the oblique pericardial sinus about the pulmonary veins and superior and inferior vena cava. (See fig. 2.)   About 250 cc. of either India Ink or Berlin Blue was injected into the right ventricle by gravity with a pressure of 60 mm. Hg.   Simultaneously, from 100 to 400 cc. of oxygenated, heparinized blood was injected into the left auricle under the same pressure.   When injections were started the ligature about the great veins was drawn tight, thereby separating the circulation of the right and left hearts.   Continuous ventricular pressures were recorded as before.   Ventricular pressures were controlled by changing the rate of the injection.   Usually right ventricular pressure was maintained below

Fig. 3   Fig. 4

Fig. 3. Section through the heart of a typical experiment done by method A, showing lack of myocardial injection.

Fig. 4. Section through the heart of a typical experiment done by method B, showing complete injection of sinusoids, venules and capillaries.

left ventricular pressure, but in a few experiments right ventricular pressure was caused to exceed left ventricular pressure.   In still other experiments these pressure relations were changed during the injection.   The hearts were stopped, fixed, and studied as before.

RESULTS.   The results of typical experiments are shown in table 1. As in method A there was no gross or microscopic myocardial capillary injection in hearts in which right ventricular systolic pressure was below left ventricular systolic pressure.   In four experiments in which the right ventricular systolic pressure was below the left ventricular systolic pressure during the first part of the injection there was no gross injection of the

hearts. In five other experiments in which the right and left ventricular systolic pressures were equal there was microscopically partial injection of venules, sinusoids and capillaries of the interventricular septum. In three of these experiments the coronary sinus was cannulated and the accessory cardiac veins were ligated to preclude any injection through the coronary veins.

When the right ventricular systolic and diastolic pressures are made to exceed the left ventricular systolic and diastolic pressures respectively

TABLE 1

EXPT. NO.	PRESSURES		REMARKS
	R. V.	L. V.	
	mm. Hg	mm. Hg	
1 (a)	28/2	95/2	Control
(b)	50/3	125/2	No gross injection
(c)	50/3	60/2	No gross injection
(d)	45/10	58/3	No gross or microscopic injection
2 (a)	26/0	100/0	Control
(b)	26/0	45/0	No gross injection
(c)	68/28	60/40	Good injection in patches of septum and right ventricle. No injection of left ventricle
3 (a)	15/0	75/3	Control
(b)	40/12	80/10	No gross injection
(c)	110/45	95/10	Heart grossly injected
(d)	85/40	48/5	Complete injection of septum, right and left ventricles grossly and microscopically
4 (a)	19/2	100/7	Control
(b)	28/3	138/3	No gross injection
(c)	50/5	75/7	No gross injection
(d)	67/10	38/8	Complete injection of septum, right and left ventricles grossly and microscopically

the picture is entirely different. Within three to four heart beats the injection material appears, first in the apical veins over the interventricular sulcus. Quickly the heart always becomes markedly discolored and remains so as long as these pressure relations are maintained. Microscopically, there is complete injection of myocardial capillaries, venules, and sinusoids in both ventricles and septum (fig. 4 and fig. 5). The left ventricular cavity usually contains a small amount of the injection material. Although the coronary veins are grossly filled with this material the coronary arteries contain only a small amount.

DISCUSSION.   Our experiments show that when India Ink or Berlin Blue is introduced into the isolated right ventricle of the beating heart, capillary injection does not occur when the right ventricular systolic pressure is below the left ventricular systolic pressure.   However, these substances produce complete capillary injection if the right ventricular systolic and diastolic pressures exceed left ventricular and diastolic pressures respectively.   That these substances are capable of producing com-
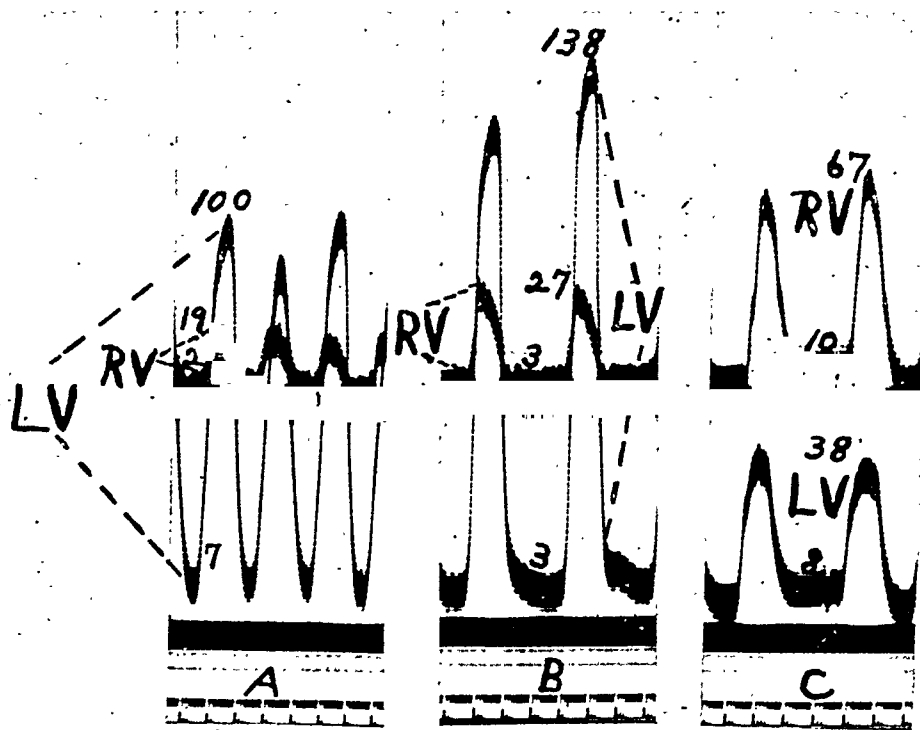
Fig. 5. Optical record of right and left ventricular pressures during a typical experiment.
A. Pressures before start of injection—heart not discolored. R.V.P.—19/2. L.V.P.—100/7.
B. During injection. R.V.P.—27/3. L.V.P.—138/3.
C. During injection—heart grossly discolored. R.V.P.—67/10. L.V.P.—38/8. R.V.P., upper curve—right ventricular pressure. L.V.P., lower curve—left ventricular pressure. Time ⅕ second.

plete injection is shown by the fact that always in method B the capillaries of the lungs were completely injected.   Also, in control experiments, the hearts were completely injected when these substances were injected into the right or left ventricle.

From our results we conclude that in the normally beating dog heart the myocardium receives no nourishment from the right ventricle through either the thebesian veins or luminal vessels.   Bohning, Jochim and Katz (5) concluded from their experiments that myocardial nourishment may

occur through thebesian veins or luminal vessels.   We believe their work does not demonstrate critically this fact for these reasons: 1, since they perfused the coronary arteries from a second dog the time and pressure relations between the ventricles and coronary arteries were not normal; 2, since they did not separate the circulations of the right and left ventricles it is impossible to say from which ventricle nourishment might occur, and 3, their published data show very little injection of the myocardial capillaries.

While it is possible that the myocardium may be nourished through thebesian veins and luminal vessels, our experiments show that if such nourishment does occur it does not have its origin in the right ventricle of the normally beating heart.

SUMMARY

The rôle of the thebesian veins and luminal vessels of the right ventricle was studied by injecting India Ink or Berlin Blue into the isolated right ventricle of the beating heart.

There was no gross or microscopic injection of the myocardium when right ventricular systolic pressure was below left ventricular systolic pressure.

Gross and microscopic myocardial injection occurred only when right ventricular systolic and diastolic pressures exceeded left ventricular systolic and diastolic pressures respectively.

Therefore, in the normally beating heart myocardial nourishment does not occur through these channels from the right ventricle.

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THE INHERENT INADEQUACIES OF THE DOUBLE HISTAMINE TEST FOR STUDIES ON PEPSIN SECRETION

GLADYS R. BUCHER AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

A lack of agreement exists regarding the output of pepsin in man and dog after histamine given subcutaneously at intervals of half to one hour. The data and conclusions of several groups of investigators (1–4) indicate that in *human subjects* there is an increased output of pepsin in response to both doses of histamine and that histamine stimulates pepsin secretion. A second group (5–9), who used *canine subjects*, obtained results that indicate that an increased pepsin output does not always occur after the second dose of histamine and that when there is an increased pepsin output after the first and occasionally after the second dose, it is to be regarded as due to a "washing out" of the pepsin in the lumen of the glands and not to stimulation of the pepsin producing cells. Before ascribing the discrepancy to a species difference, it was decided *a*, to investigate the validity of existing data on the response of dogs to the double histamine test, using new techniques; *b*, to learn whether artifacts in the pepsin response occur due to an unfavorable pH of the gastric juice at the time of collection, and *c*, to determine whether "washing-out" processes constitute an actual complication in this test. The new techniques to which we refer are 1, the hemoglobin method for pepsin determination, and 2, the use of dogs prepared surgically with vagotomized pouches of the entire stomach.

PROCEDURE AND METHODS. *The double histamine test.* Dogs prepared with vagotomized pouches of the entire stomach were the subjects. All food was withheld for 18 hours previous to an experiment. After draining any residual juice, the continuous secretion over two 1-hour periods was collected for control purposes. Each dog then received subcutaneously 1 mgm. of histamine dihydrochloride freshly dissolved in physiological saline. Collections were made into graduated centrifuge tubes every 15 minutes for an hour. At this time the dose of histamine was repeated and the collections continued for 1.25 hours.

All samples were analyzed individually for pepsin, free and total acidity. Pepsin was determined by the Beazell modification of the hemoglobin method (10) first described by Anson and Mirsky (11) and used by Nor-

throp (12) in his studies on crystalline pepsin. It has the advantage of being very sensitive and is more reliable than any other method employed in our laboratory. All determinations were made in duplicate, accompanied by a blank test on the inactivated juice. Since preliminary tests showed that gastric juice does not lose any peptic activity if stored in the ice box for several months, our samples were stored overnight in the ice box and the pepsin determinations made the next day. Free and total acidity were determined by titration with N/40 NaOH using Töpfer's and phenolphthalein as indicators.

Total pepsin output has been the major consideration in this work. It is the product of pepsin concentration and the volume, thus representing both variables. The results are expressed graphically as the mean curves of the arithmetical averages on the individual animals. We have preferred to use the milli-unit when expressing concentration as it enables us to express the activity per cubic centimeter as a whole number. All important trends shown by the data were investigated for statistical significance. The criterion of significance was a critical ratio of at least 3.

Method for studying effect of pH on peptic activity in gastric juice. Dog gastric juice collected under conditions of uniform secretion at pH 0.9 to 1.3 and known to have a good peptic activity was used. Aliquots were exposed for exactly 15 minutes to various pH values by diluting with an equal volume of 0.1 N HCl, water, or diluted 0.1 N NaOH. The exposure was terminated by starting the pepsin determination using 1 cc. of the diluted gastric juice in 5 cc. of the hemoglobin substrate. The pH of the digestion mixture was thereby not significantly altered from 2.2 (Coleman glass electrode). The activity manifest at the various pH values was related to that when water or 0.1N HCl was used as the diluent.

Procedure for studying possibility of "washing out." After obtaining two 1-hour samples of basal secretion, continuous histamine stimulation was inaugurated, wherein each animal received a small constant dose of histamine every 10 minutes. After 2 hours, during which a uniform secretory rate had become established, a single large dose equivalent to 7 of the 10-minute doses, was given all at once. No more histamine was given until an hour later, when the continuous stimulation was resumed and continued for another hour. The gastric juice was collected for analysis at 20-minute intervals.

DATA AND DISCUSSION. *Can it be demonstrated that in the dog there is an apparent increased output of pepsin in response to repeated hourly injections of histamine?* The results of 24 double histamine experiments, representing 6 on each of 4 animals, appear as the mean curves of arithmetical averages in figures 1 and 2. The concentration of pepsin as well as the output of pepsin *increased* in response to *both* injections of histamine. When the total hourly outputs of pepsin are studied (fig. 3) those following

both histamine injections are significantly larger than the control. Statistical analysis demonstrates a significant homogeneity between the responses of both experimental hours and again between those of the control hours.

Comment. Thus our findings with respect to the dog confirm those of Alley (7) and bring the observation in man and dog·into mutual agreement. Analysis of the data of Vineberg and Babkin (6) and Toby (9) reveals that they were considering pepsin concentration only. If the pepsin output is calculated from their data, their findings are in agreement with ours. Bowie and Vineberg's work (8) with anesthetized dogs is also

COMPOSITE OF AVERAGE RESPONSES
IN FOUR ANIMALS (24 EXP.)

Series A — Double histamine
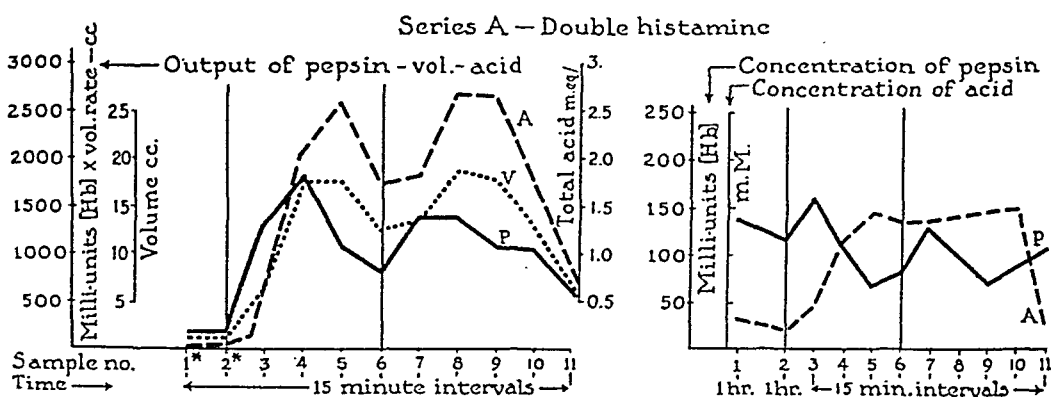
Fig. 1. Fig. 2.

Fig. 1. The average output responses under the double histamine test. *A* = acid output in milli-equivalents; *V* = volume output in cubic centimeters gastric juice; *P* = pepsin output in milli-units pepsin (Hb). Vertical lines at 2 and 6 indicate time at which 1 mgm. histamine was injected. * denotes values calculated to a fifteen minute basis from the hourly data.

Fig. 2. Average curves representing concentration of pepsin, *P,* in milli-units (Hb) per cubic centimeter and concentration of acid, *A,* in milli-equivalents per liter, obtained in response to the double histamine test.

in agreement if the hourly outputs for the first 2 hours only are compared. In 10 experiments, Gilman and Cowgill (5) failed to observe any consistent increase in pepsin output in response to the second injection of histamine. This is not supported by Goodman (15) or ourselves.

The greater number and uniformity of these data, however, neither establish nor invalidate either interpretation (stimulation vs. mechanical washing-out) of the mechanism by which histamine supposedly influences pepsin output. The nature of this test neither proves that washing-out processes are an actuality, nor excludes such from entering into the picture during the second hour as a result of the retarded flow at the end of the first. Moreover the statistical homogeniety of the significantly increased

·total hourly outputs during the experimental hours can be viewed from at least two other aspects which are exclusive of a washing-out process being concerned. These aspects are *a*, that the chief cells have been more productive and to an equally intensive degree during both experimental hours; or *b*, that the low basal outputs of the basal secretion are artifacts due to inactivation of the pepsin that is continually being secreted.

Is it possible that in low gastric acidities considerable pepsin is inactivated during the periods of collection? It is well known (13, 14) that pepsin, either in the gastric juice or in active mucosal extracts, is rapidly and irreversibly destroyed by titration to neutrality. It has not been heretofore realized that pepsin, as it occurs in the gastric juice is more severely in-
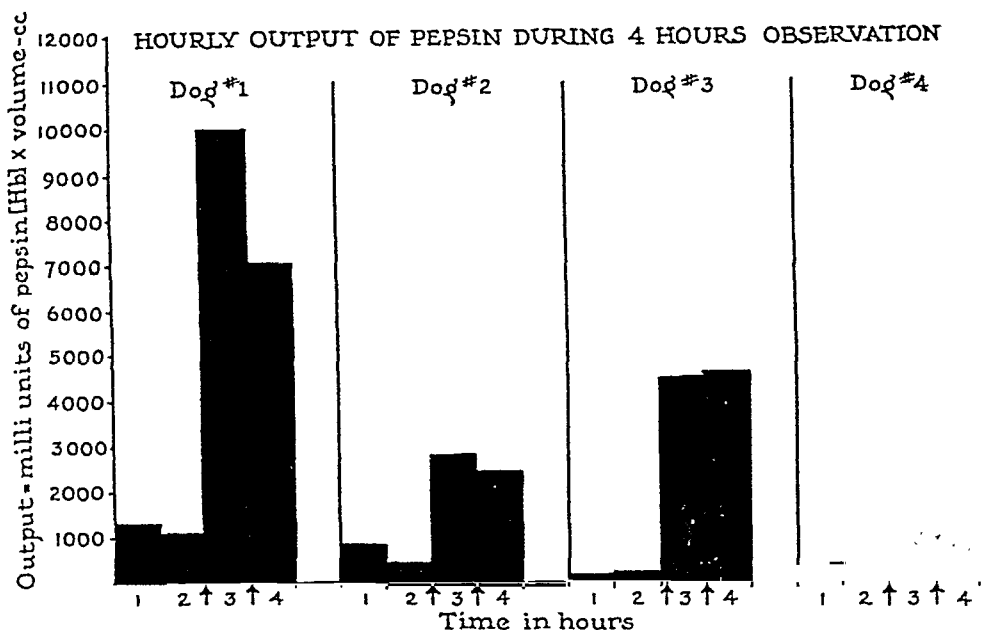
Fig. 3. Total hourly pepsin outputs of four dogs obtained under double histamine stimulation. Hours 1 and 2 are control hours. Arrows indicate the time of injection of 1 mgm. of histamine.

activated by pH changes in the range between 2.0 to 4.0 than in the range between pH 4.0 to 8.0.

The results of 38 experiments on gastric juice from 3 different dogs are presented in figure 4. In every instance a marked loss of activity was noted when the exposure was still as strongly acid as pH 2.0, amounting to approximately 30 per cent. At pH 3.5, which with Töpfer's still indicates the presence of some free acidity, the loss of peptic activity was about 60 per cent of the original. We believe that this evidence points to the necessity of collecting gastric juice intended for pepsin study under conditions where a pH of 0.9 to 1.3 is sure to prevail at all times. As soon as the pepsin is exposed to a pH higher than this range, reductions in the mani-

fest activity are invited, and the physiological efforts of the chief cells with regard to peptic output will be grossly underestimated.

That such conditions do not prevail in the double histamine test is demonstrated by the acidity curve in figure 2. If it were possible to obtain the pH on the scanty basal secretions and to know the time any given fraction of pepsin had been exposed to that pH, it is conceivable that the difference between the basal output and histamine output might be completely obliterated.

Can washing-out processes be demonstrated to be possibly involved in the double histamine test? The results of one experiment on each of 4 dogs is given in the form of average curves in figures 5 and 6, and as average hourly outputs in figure 7. It can be seen that under continuous histamine stimulation there is an induction period of one hour, after which 6 basal
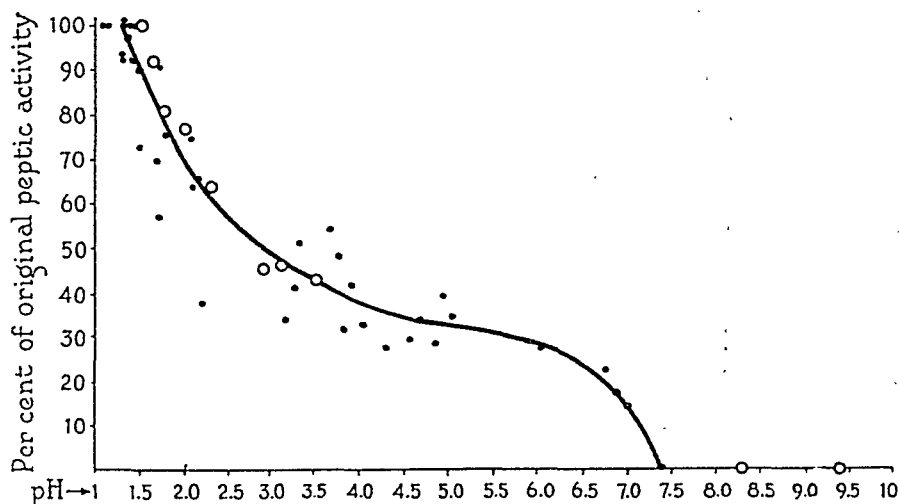
Fig. 4. Curve showing loss of peptic activity with increasing pH. Circles represent points obtained by using U.S.P. pepsin powder (Merck). The solid points are the values obtained by using dog's gastric juice.

doses, given one every 10 minutes, maintained a uniform secretory rate of uniformly high acidity with a remarkably constant total pepsin output. The response to 7 times the basal dose given as one dose at the start of the fifth hour was a decreased hourly output of *both* volume and pepsin. The volume-rate and pepsin output per minute had become reduced to half by the end of the hour. The resumption of the continuous histamine not only revived the secretory rate, but brought forth a clearly increased pepsin output. However, examination of the hourly outputs (fig. 7) supports the view that this augmented output may be the result of pepsin accumulation in the preceding hour due to the decreased volume-rate at the end of that hour. The average hourly output under continuous histamine (hrs. 3 and 4) was 4511 milli-units; that of the fifth and last hours was 4639 milli-units. These very similar averages speak for themselves.
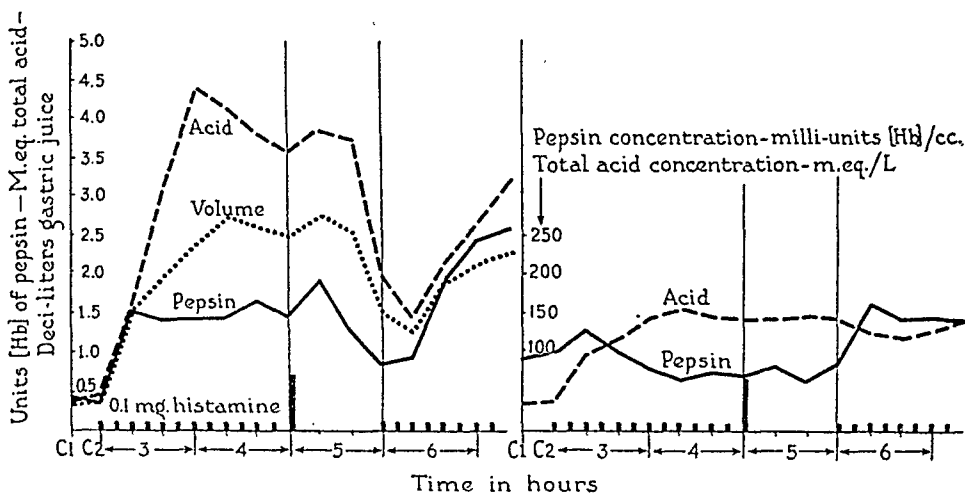
Fig. 5   Fig. 6

Fig. 5. Average total output curves of acid, volume and pepsin under continuous histamine (hrs. 3, 4 and 6) and under single histamine (hr. 5). Acid is expressed in milli-equivalents; volume as deciliters; pepsin as units (Hb). Small bars along the bottom indicate time and size of histamine dose.

Fig. 6. Average concentration curves of acid and pepsin during continuous histamine (hrs. 3, 4 and 6) and single histamine (hr. 5). Acidity is expressed as milli-equivalents per liter; pepsin is expressed as milli-units (Hb) per cubic centimeter.
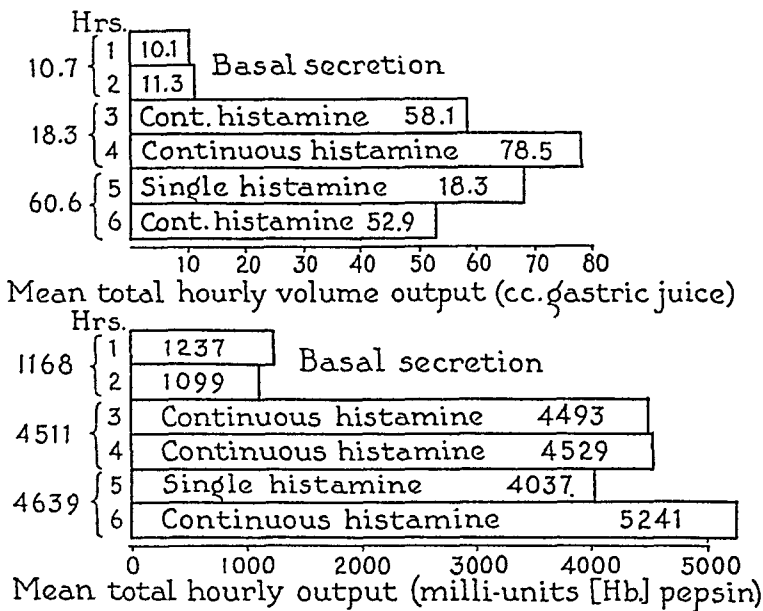
Fig. 7. Average total hourly outputs of volume in cubic centimeters gastric juice (upper) and average total hourly output of pepsin in milli-units (Hb) (lower) under continuous histamine (hrs. 3, 4 and 6) and single histamine (hr. 5).

SUMMARY. We believe that the lack of agreement regarding the interpretation of the effect of histamine on pepsin response has its basis in

the inadequacies of the experimental approach rather than in errors or insufficiencies of the data obtained therefrom. We have found that partial inactivation of pepsin must be expected when the latter is secreted with gastric juice of low acidity, as occurs in several phases of the double histamine test. Because all deductions relative to the *amount* of pepsin liberated are made on the basis of its manifest activity at the time of collection, this factor is of great importance in the quantitative evaluation of chief cell activity. Also, complications exist which appear to be the result of accumulation and subsequent washing-out and which are unavoidable when single hourly doses of histamine are given. The double histamine test therefore has no utility in the quantitative study of pepsin secretion; this is especially true when the question of whether histamine stimulates pepsin-cell activity is involved.

CONCLUSIONS

1. In dogs with a vagotomized pouch of the entire stomach the hourly injection of histamine, or the application of the double histamine test, increases the output of pepsin apparently.

2. For the purpose of determining whether histamine stimulates pepsin production or only mechanically promotes the elimination of preformed pepsin such evidence is completely indifferent.

3. Two limitations of the double histamine test for the quantitative study of pepsin secretion have been detected. They are: *a*, artifacts due to pH inactivation occur in gastric juice collected at low and irregular acidity which are still acid to Töpfer's reagent; and *b*, artifacts due to the possibility of a washing-out process are unavoidable when great irregularities in the gastric secretory rate occur as they do in the double histamine test.

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THE STORAGE OF CARBOHYDRATE FOOD

EATON M. MacKAY AND DOUGLAS R. DRURY

*From The Scripps Metabolic Clinic, La Jolla and The Department of Physiology,
University of Southern California Medical School, Los Angeles*

The largest proportion of the calories comprising the diet of man, most rodents and all herbivorous animals is in the form of carbohydrate. The ability of the mammalian organism to store carbohydrate as such is very limited. No one would deny that carbohydrate food in excess of the requirement over relatively long periods is stored as fat but the idea is prevalent throughout the metabolic literature that oxidation and deposition as glycogen accounts for almost all of the carbohydrate food ingested under ordinary circumstances. This is true for the conditions under which Cori and Cori (1) carried out their balance studies. They made their observations over the short period of 4 hours and administered sugar under conditions (2) which give absorption rates less than half those which are possible under more natural conditions (3). There are many circumstances under which even a rough calculation will show that there is an excess of ingested carbohydrate which cannot be promptly catabolized or retained as such and which therefor must be stored as fat. This formation of fat from carbohydrate is a normal process in all well fed animals when their glycogen stores become filled. Burr and his co-workers (4, 5) have presented evidence obtained with the respiratory quotient for carbohydrate storage as fat in normal rats and in a more marked degree in rats suffering from a deficiency of the essential fatty acids. Except for this we have little information as to factors which may influence the amount of ingested carbohydrate stored as fat and no direct evidence for such storage. The present study was undertaken to obtain data by direct measurements on the extent of the storage of carbohydrate food as fat over a relatively short period of time.

METHODS. The method devised to approach this problem consisted in taking pairs of litter-mate female rats and feeding and fasting them on alternate days until their food intake became approximately constant on the alternate feeding days and the respective fed and fasted weights were constant. A typical example is shown in figure 1. The food cups were left in the cages for 20 of the 24 hours during the feeding periods so that the fed weights were 4 hours postabsorptive. After equilibrium in weight changes had been established one rat of each pair was sacrificed at the end

of a feeding period (4 hrs. post-absorptive) and the other at the end of a fasting period.   An experiment of this nature presents difficulties not found in the study of glycogen deposition due to the variability in the amount of preformed fat in the organism.   The litter mate pairs selected in the first place were always of the same weight.   At the termination of the experiment only those pairs were retained the fasting weights of which were essentially the same.   This led to far more data being discarded than was retained.   Ten pairs of the rats used in the data presented here were 129 days old when sacrificed and 6 pairs were 137 days of age.   All of them were on the regime depicted in figure 1 for the 20 day period.   Six pairs were fasted 24 hours, 6 pairs for 48 hours and 4 pairs for 60 hours before pairing on the basis of body weight and the first feeding of the special diet.   The
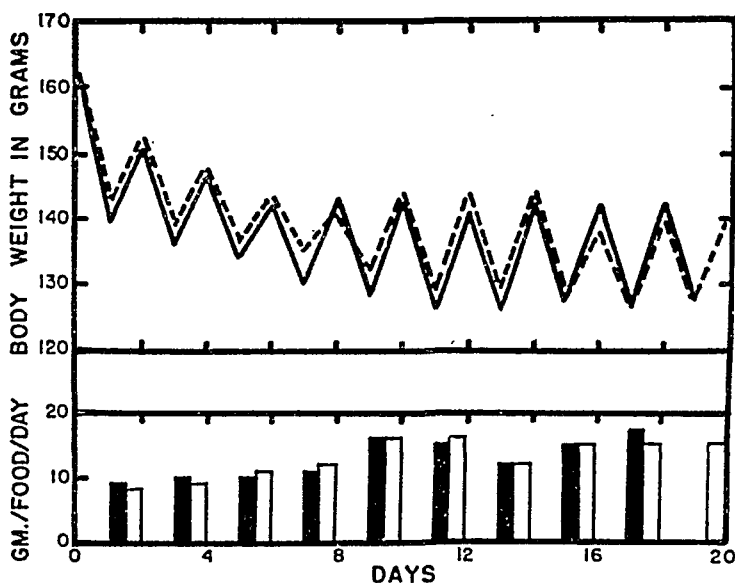
Fig. 1. Food intake on feeding days and daily variation in body weight of a typical pair of rats on regime of alternate fasting and feeding.

48 hour fast gave the best results.   After fasts of 100 or more hours which might more nearly reduce the body fat to a more regular basal level the special diet is eaten poorly and the rats continue to lose weight and die during the ensuing regime.

The special diet which we used may be considered to be fat free for our purposes.   It consisted of sucrose 70, casein (vitamin free, Casein Company of America) 15, brewer's yeast (Anheuser-Busch) 10 and Osborne and Mendel's (6) salt mixture 5.   Vitamins A and D were supplied by feeding a small drop of a natural fish liver oil concentrate every third day.   In our rough calculations of the carbohydrate intake the sucrose was considered as such.   Fifty-two per cent of the yeast was taken as protein and it was assumed that 60 per cent of this and of the casein was

converted to carbohydrate although the correct figure may be higher (7). No attention was paid to the extremely small lipid content of the casein or yeast or to the possibility of fat formation from the fed protein.

TABLE 1

Relative storage as glycogen and fat of carbohydrate food

(Averages of 16 pairs of rats on alternate fasting and feeding regime)

	FASTED*			FED†		
	Min.	Av.	Max.	Min.	Av.	Max.
Initial body weight—grams...	136	*169*	195	135	*169*	194
Final body weight:						
Fed—grams................	125	*150*	178	130	*155*	177
Fasted—grams............	112	*134*	157	116	*137*	156
Food intake—grams/rat/day‡.	13	*17*	21	13	*17*	21
Body glycogen:						
Per cent..................	0.15	*0.19*	0.24	0.32	*0.63*	1.27
Grams/rat................	0.18	*0.26*	0.33	0.44	*0.98*	1.84
Body fat:						
Per cent..................	1.60	*3.54*	7.05	2.50	*5.10*	8.88
Grams/rat	2.06	*4.75*	9.04	4.38	*7.92*	13.58

* One of each pair sacrificed at end of fasting day 28 hours after food had been removed from their cages.

† Second rat of each pair sacrificed at the end of a feeding day 4 hours after they had last had access to food.

‡ This is the food intake on the feeding days which of course serves a two day period.

Calculations from above data

Body weight gain during feeding days......................	18 grams
Food intake as grams per rat per day......................	8.5 grams
Food intake as calories per rat per day....................	33 Calories
Carbohydrate intake as grams per rat per day..............	7 grams
Carbohydrate intake as calories per rat per day............	29 Calories
Carbohydrate deposited as glycogen per rat................	0.72 ±.08* gram
Carbohydrate deposited as glycogen per rat................	3 Calories
Carbohydrate deposited as fat per rat	3.17 ±0.55* grams
Carbohydrate deposited as fat per rat	29 Calories
Carbohydrate calories deposited as glycogen...............	10 per cent
Carbohydrate calories deposited as fat.....................	100 per cent

* Standard error.

When the rats were to be sacrificed they were anesthetized with an intraperitoneal injection of a solution of sodium pentabarbital, the abdomen and thorax split open and the animal quickly liquidized in a hot 30 per cent KOH solution using 3 cc. of this per gram of rat. The bones are

easily filtered off with the aid of glass wool. The glycogen content of the body was found from the glycogen concentration of this solution determined in the usual manner (8). Body "fat" was determined as the petroleum ether extract of an acidified aliquot of the KOH solution and consisted of unsaponifiable lipids and fatty acids. All of the increase in the animals showing fat storage was in fatty acids.

RESULTS. Our average figures are presented in table 1. For every pair of rats the amount of glycogen and fat in the animal was greater in the fed rat.

The food intake per rat per day in the table represents the average intake on the last four feeding days which of course cover an eight day period. The food intake per rat per day used in the calculations below this table is one-half of this figure for the last day for the *fed* group. It is just a coincidence that the averages for the last four feeding days of both groups and the last day of the *fed* group are the same. We assume that the food intake of the "feeding" day which serves for this day and the "fasting" day which follows is utilized equally during each of these days. Actually the energy expenditure is probably larger during the "feeding" day (5) but this difference would not significantly alter our calculations.

Both glycogen and fat are obviously stored after the largely carbohydrate diet (table 1). Of the two, storage as fat is by far the more important. Below table 1 calculations have been made from our average data. One should not be misled as to the accuracy of experiments of this type by the fact that our averages happen to show that we can account for all of the carbohydrate calories which are stored. Of much more importance is the ratio of carbohydrate stored as glycogen to that as fat which is 1:10.

DISCUSSION. Under the conditions of our experiments carbohydrate food is stored chiefly as fat and only in a minor degree as carbohydrate (glycogen). We do not pretend that this must always be the case in the rat for this animal normally eats during the night and fasts during the day. In order to have suitable quantities of food, body fat and glycogen to deal with we found it necessary to double these periods. The metabolism associated with preparation for a longer fasting period may be somewhat different than with the usual 12 hour schedule but it would be surprising if much more of the stored carbohydrate was in the form of glycogen. If glycogen storage was the same there would still be 4 or 5 times as much of the carbohydrate food stored as fat as in the form of carbohydrate (glycogen). It is possible that this type of experiment might be refined so that observations could be made over the normal feeding schedule of the rat. In any case our results show the importance of transient or labile carbohydrate storage as fat and give a basis for a study of the influence of various factors upon carbohydrate storage.

SUMMARY

When rats are fed and fasted on alternate days so that their average weight and food intake becomes constant sufficient food is stored during the feeding day for maintenance during the fasting day. If the diet is composed almost entirely of carbohydrate this is largely stored in the form of fat.

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PROTHROMBIN AND FIBRINOGEN IN LYMPH[1]

K. M. BRINKHOUS AND S. A. WALKER

From the Department of Pathology, State University of Iowa, Iowa City

It has been a common observation that lymph collected from different sources clots on standing—an observation which indicates that all the factors necessary for clotting, including prothrombin, are present in lymph. However, we are aware of only one study of prothrombin in lymph—that of Howell (1)—in which it was demonstrated that this clotting factor is present in thoracic duct lymph of dogs. No data regarding the quantity of prothrombin in lymph are available.

In this investigation we have determined quantitatively the prothrombin content of dog lymph collected from several sites, namely, thoracic duct, portal lymphatics draining the liver, and femoral lymphatics. Our results show that prothrombin is present in lymph in considerable amounts, but in a lower concentration than in plasma. The distribution of this clotting factor between lymph and plasma is in general the same as the distribution of the total proteins between lymph and plasma.

In these experiments lymph was collected from fasting dogs except in three instances (dogs 1, 12 and 21); ordinarily the last feeding was 24 to 48 hours prior to the time of collection. Ether anesthesia was used in all experiments. Each animal was given 1 mgm. morphine sulfate per kilogram body weight subcutaneously $1\frac{1}{2}$ to 2 hours prior to the beginning of anesthesia. A short segment of the lymphatic from which lymph was to be collected was isolated and divested carefully of adjacent capillaries and other tissue. The vessel was then washed thoroughly with 0.9 per cent NaCl solution. The lymphatic was incised, and the lymph collected in a small paraffin-coated spoon which contained a measured amount of potassium oxalate solution (1.85 per cent). In the case of femoral lymph, the flow was stimulated by passive motion of the leg for a period of approximately 10 minutes. The first few drops of lymph collected after beginning the motion were discarded.

Blood samples were obtained from each dog by venipuncture (jugular). The blood (7 cc.) was mixed immediately with potassium oxalate (1 cc.)

and the plasma, obtained by centrifugation, was used for comparison with the oxalated lymph samples from the same dog.

Prothrombin analyses were made immediately on all samples by the two-stage method of Warner, Brinkhous and Smith (2, 3). The prothrombin levels of lymph and plasma were determined simultaneously. The result of each lymph prothrombin determination is expressed in per cent, using the same dog's plasma prothrombin content as 100 per cent. In addition to these quantitative prothrombin determinations, the prothrombin level of thoracic duct lymph was measured by the one-stage method of Quick (4) in 4 of the dogs (dogs 9, 13, 16 and 17, table 1). Fibrinogen determinations were made on a few of the plasma and thoracic duct lymph samples. The method of Jones and Smith (5) was used.

The *results* obtained are shown in table 1. For thoracic duct lymph, the mean prothrombin level and the mean fibrinogen level were both approximately one-half the plasma levels of these clotting factors. The average prothrombin value of liver lymph (93 per cent) was considerably higher than in the case of thoracic duct lymph, while the average value for femoral lymph (7.6 per cent) was very much less.

It will be noted from table 1 that there are wide variations in the prothrombin content of thoracic duct lymph in different animals. The lowest value is 32 per cent, the highest 94 per cent. Similar differences will be noted in the prothrombin values based on Quick's clotting time. These variations are in sharp contrast to the relative constancy of the prothrombin level of normal dog plasma (2). A possible explanation of these differences in prothrombin level of thoracic duct lymph is suggested by the fact that it is made up of a combination of various lymphs (i.e., portal, femoral, etc.), the prothrombin content as well as the volume of which are unequal, and probably variable from time to time.

It is well established that the total protein content varies considerably in lymph from different portions of the body. In general, the protein in liver lymph is highest, that in leg lymph lowest, with average values of about 85 per cent and 28 per cent respectively of the plasma protein content. The protein in thoracic duct lymph is intermediate in amount, with average values of about 55 per cent (6, 7, 8). If one compares these values with the relative values of prothrombin and fibrinogen in lymph given in table 1, a remarkably close correspondence between them will be noted. This suggests that the process by which prothrombin and fibrinogen reach the lymph is the same as for the other lymph proteins. In the case of liver lymph, the high protein values have been attributed to the ready permeability of the liver sinusoids, allowing the rapid diffusion of the plasma into the tissue fluid and lymph in this organ. On the other hand, there is much evidence to indicate that both prothrombin (9) and fibrinogen, and probably other plasma proteins (10), are manufactured in

the liver, and the high prothrombin and high protein contents of liver lymph may be related to the hepatic formation of these substances. However, studies (8) with one of the colloidal dyestuffs, brilliant vital red, injected intravenously, have shown that the distribution of the dye between plasma and lymph is in general the same as the distribution of the total proteins. These data would suggest that the liver sinusoids are

TABLE 1

Prothrombin and fibrinogen in lymph

ANIMAL NO.	THORACIC DUCT LYMPH				PORTAL LYMPH	FEMORAL LYMPH
	Fibrinogen		Prothrombin (two-stage method)	Prothrombin (one-stage method)	PROTHROMBIN (TWO-STAGE METHOD)	PROTHROMBIN (TWO-STAGE METHOD)
	mgm. per 100 cc.	*per cent**	*per cent†*	*per cent†*	*per cent†*	*per cent†*
1			33.0			
2			32.0			
3					90.0	9.3
4					125.0	
5			50.0			
6					100.0	
7	164	43.0	59.0			
8			34.5			
9	236	50.0	41.0	23.0		5.8
10						7.5
11					82.0	
12	145	40.0	61.0			
13			43.0	28.0		
14						7.8
15					67.0	
16	259	60.0	57.0	55.0		
17	362	71.0	94.0	66.0		
18					95.0	
19	130	53.0	52.0			
20	169	51.0	65.0			
21	·224	42.0	37.0			
Average..	211	51.2	50.7	43.0	93.2	7.6

* Plasma fibrinogen = 100 per cent.
† Plasma prothrombin = 100 per cent.

about equally permeable to the dye and proteins, and that the permeability factor is probably more important than local protein formation in determining the high protein content of liver lymph.

SUMMARY

Prothrombin and fibrinogen are present in thoracic duct lymph of dogs in a concentration equal to approximately one-half the plasma concentra-

tions of these substances. The prothrombin content of liver lymph is nearly equal to that of blood plasma, while femoral lymph contains less than one-tenth as much prothrombin as plasma.

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A COMPARISON OF THE EFFECTS OF 11-DESOXYCORTICO-STERONE ACETATE AND 17-HYDROXY-11-DEHYDRO-CORTI-COSTERONE IN PARTIALLY DEPANCREATIZED RATS[1]

DWIGHT J. INGLE and GEORGE W. THORN

From the George S. Cox Medical Research Institute, University of Pennsylvania and the Chemical Division, Medical Clinic, The Johns Hopkins University and Hospital

Among the principal physiologic effects of 11-desoxycorticosterone are its efficacy in maintaining the life of adrenalectomized animals (1), (2), and its influence in regulating electrolyte balance in normal as well as adrenalectomized animals (2), (3). The effect of this compound on carbohydrate metabolism is reported to be slight (4), (5), (6). In contrast, 17-hydroxy-11-dehydro-corticosterone which appears to be less effective in maintaining the life of adrenalectomized animals (7), is very potent in its effect on the carbohydrate metabolism of both normal and adrenalectomized animals (4), (5), (6), as well as patients with Addison's disease (8). The deduction has been made that these two substances differ in their principal physiologic properties. In the present investigation an attempt has been made to extend this comparison by studying the physiological changes which occurred in partially depancreatized and in adrenalectomized, partially depancreatized rats following treatment with one or the other of these crystalline adrenal cortical hormones.

METHODS. Male albino rats of the Sprague-Dawley strain were used in these experiments. The animals were partially depancreatized at a body weight of 45 to 80 grams, all of the pancreas being removed except that portion which lay between the bile duct and the duodenum. The animals were maintained on a diet of Purina Dog Chow until they had reached a weight of approximately 300 grams. They were then placed in metabolism cages and maintained on a fluid diet composed of butter 500 grams, dried egg albumin 170 grams, sucrose 325 grams, Celluflour 83 grams, Osborne and Mendel salt mixture 33 grams, sodium chloride 17 grams, Vi-Penta (Roche) 10 cc., with water added to make a total volume of 1680 cc. The diet was administered by stomach tube each morning and evening, the total daily amount being 18.4 cc. per rat. The steroid compounds were administered in sesame oil by subcutaneous injection.

Twenty-four-hour specimens of urine were collected and preserved with toluene. The methods used for the analysis of urine have been described (2).

In the first series of experiments 8 partially depancreatized rats were used. Following a control period of 7 days, 11-desoxycorticosterone acetate was administered for 4 days to 5 of the animals and after a second control period of 7 days a similar quantity of 17-hydroxy-11-dehydrocorticosterone was administered for 4 days to the same animals. Two of the remaining 3 animals received only 17-hydroxy-11-dehydrocorticosterone and one animal received only 11-desoxycorticosterone acetate.

In the second series of experiments 10 adrenalectomized, partially depancreatized rats were used. Following operation the animals were permitted food ad libitum for a period of at least 4 days before force-feeding was resumed. For one week following operation, maintenance doses of aqueous adrenal cortical extract were injected and 1 per cent solution of sodium chloride was given as drinking water. Each animal was then maintained for a period of 6 or 7 days without treatment prior to the beginning of the test period. Nine of the 10 partially depancreatized animals were observed to have glycosuria prior to adrenalectomy. In all of the diabetic animals glycosuria was abolished by adrenalectomy. Six animals were treated with 11-desoxycorticosterone acetate for 4 days and, after a control period of 7 days, they were then treated with a similar quantity of 17-hydroxy-11-dehydrocorticosterone for 4 days. Two animals received only 17-hydroxy-11-dehydrocorticosterone and two animals received only 11-desoxycorticosterone acetate.

RESULTS. *Body-weight.* A striking difference was observed in the effect of the two test substances (fig. 1). All of the partially depancreatized and all of the adrenalectomized, partially depancreatized rats continued to gain weight during treatment with 11-desoxycorticosterone acetate whereas these same animals lost weight during the period in which they were treated with 17-hydroxy-11-dehydrocorticosterone. Most of the rats succumbed during, or as a result of, treatment with 17-hydroxy-11-dehydrocorticosterone and at the time of death these animals appeared to be markedly dehydrated.

Excretion of glucose and non-protein nitrogen. The daily administration of 1, 2 or 5 mgm. of 11-desoxycorticosterone acetate failed completely to induce glycosuria in either the partially depancreatized or the adrenalectomized, partially depancreatized rats. Furthermore, no increase in the renal excretion of non-protein nitrogen was noted during the period of treatment with this compound. It is of some interest to note that in the majority of animals the excretion of non-protein nitrogen was somewhat reduced (approximately 6 per cent) during and immediately (4 days) following 11-desoxycorticosterone acetate therapy. Three animals (one

partially depancreatized, and two adrenalectomized, partially depancre-
atized) were treated with larger doses of 11-desoxycorticosterone acetate
(10 mgm. per day). In two of these animals so treated (one partially
depancreatized and one adrenalectomized, partially depancreatized) a
slight increase in glucose and nitrogen excretion was noted (table 1).

The daily administration of 1, 2 or 5 mgm. of 17-hydroxy-11-dehydro-
corticosterone was followed by a marked glycosuria and a definite increase
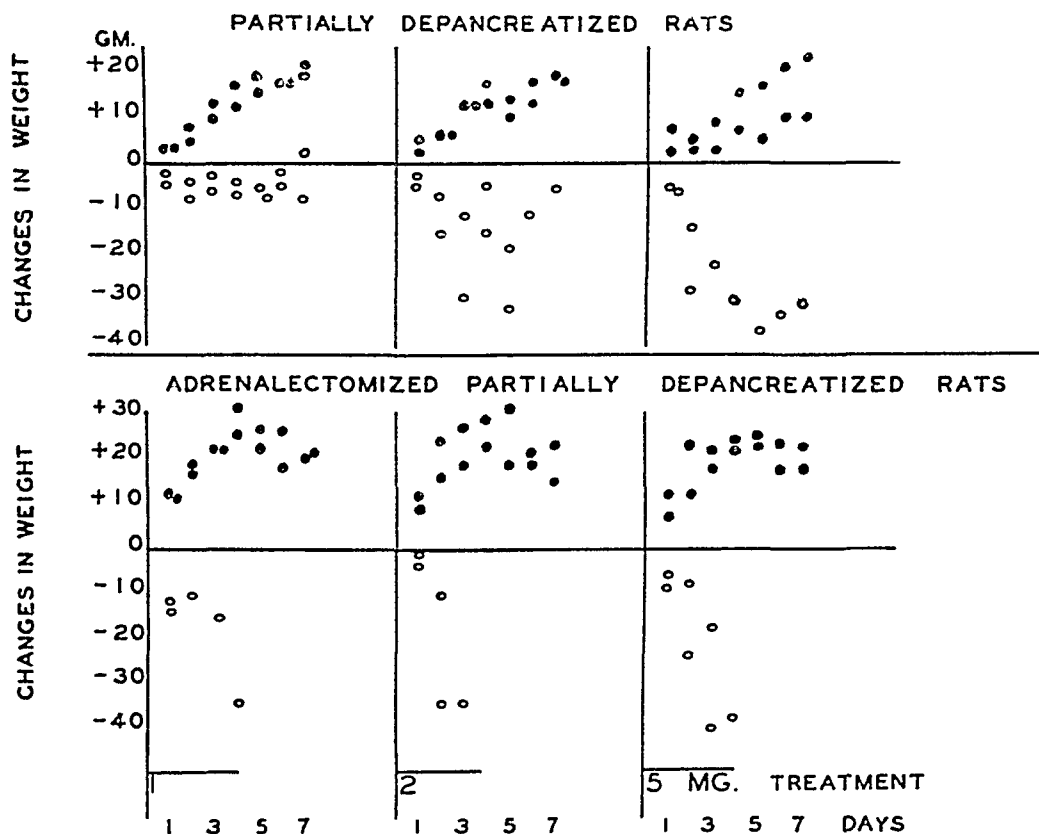
Fig. 1. A comparison between the effect of 11-desoxycorticosterone acetate and
17-hydroxy-11-dehydrocorticosterone treatment on body-weight. ●—treated with
11-desoxycorticosterone acetate; ○—treated with 17-hydroxy-11-dehydrocorti-
costerone.

in the excretion of non-protein nitrogen in both the partially depancre-
atized and the adrenalectomized, partially depancreatized animals (fig. 2).
However, all of the increased glucose excretion could not be accounted
for by the increase in formation of glucose from protein on the basis of a
glucose:nitrogen ratio of 3.6 (6).

Excretion of ketone bodies. Ketonuria accompanied glycosuria and in-
creased non-protein nitrogen excretion in three animals treated with 17-
hydroxy-11-dehydrocorticosterone (table 2). One of these animals was

TABLE 1

The effect of treatment with large doses of 11-desoxycorticosterone acetate (10 mgm. daily) on glucose and non-protein nitrogen excretion averages

ANIMAL	PERIOD (4 DAYS EACH)	BODY WEIGHT	GLUCOSE	NON-PROTEIN NITROGEN
		grams	*grams/day*	*gram/day*
Partially depancreatized (rat 8)	Control	353	0.9	0.186
	Treated	340	3.1	0.254
	Control	366	0.0	0.187
Adrenalectomized, partially depancreatized (rat 24)	Control	326	0.0	0.145
	Treated	342	0.0	0.170
	Control	352	0.0	0.145
Adrenalectomized, partially depancreatized (rat 25)	Control	312	0.0	0.196
	Treated	337	2.2	0.202
	Control	340	0.0	0.189

TABLE 2

The effect of treatment with 17-hydroxy-11-dehydrocorticosterone on the excretion of ketone bodies

ANIMAL	HORMONE	KETONE EXCRETION, MGM. PER DAY				
		1	2	3	4	5
A. Partially depancreatized						
	mgm. per day					
Rat 3.................	1	0	0	0	0	0
Rat 17.................	1	0	0	0	0	0
Rat 3.................	2	0	0	0	0	0
Rat 15.................	2	0	10	41	0	138*
Rat 3.................	5	0	0	0	0	0
Rat 20.................	5	0	0*			
B. Adrenalectomized, partially depancreatized						
Rat 21.................	1	0	0*			
Rat 17.................	1	0	0	0	0*	
Rat 23.................	2	0	0	0*		
Rat 14.................	2	0	0*			
Rat 22.................	5	0	54	262*		
Rat 19.................	5	0	0	0	176	110*

* Animal succumbed.

partially depancreatized and two were adrenalectomized, partially depancreatized. Prior to death the carbon dioxide combining power of the blood of two of the animals was observed to be 17 and 24 volumes per

cent, respectively. These data suggest that diabetic acidosis was the cause of death in these animals.
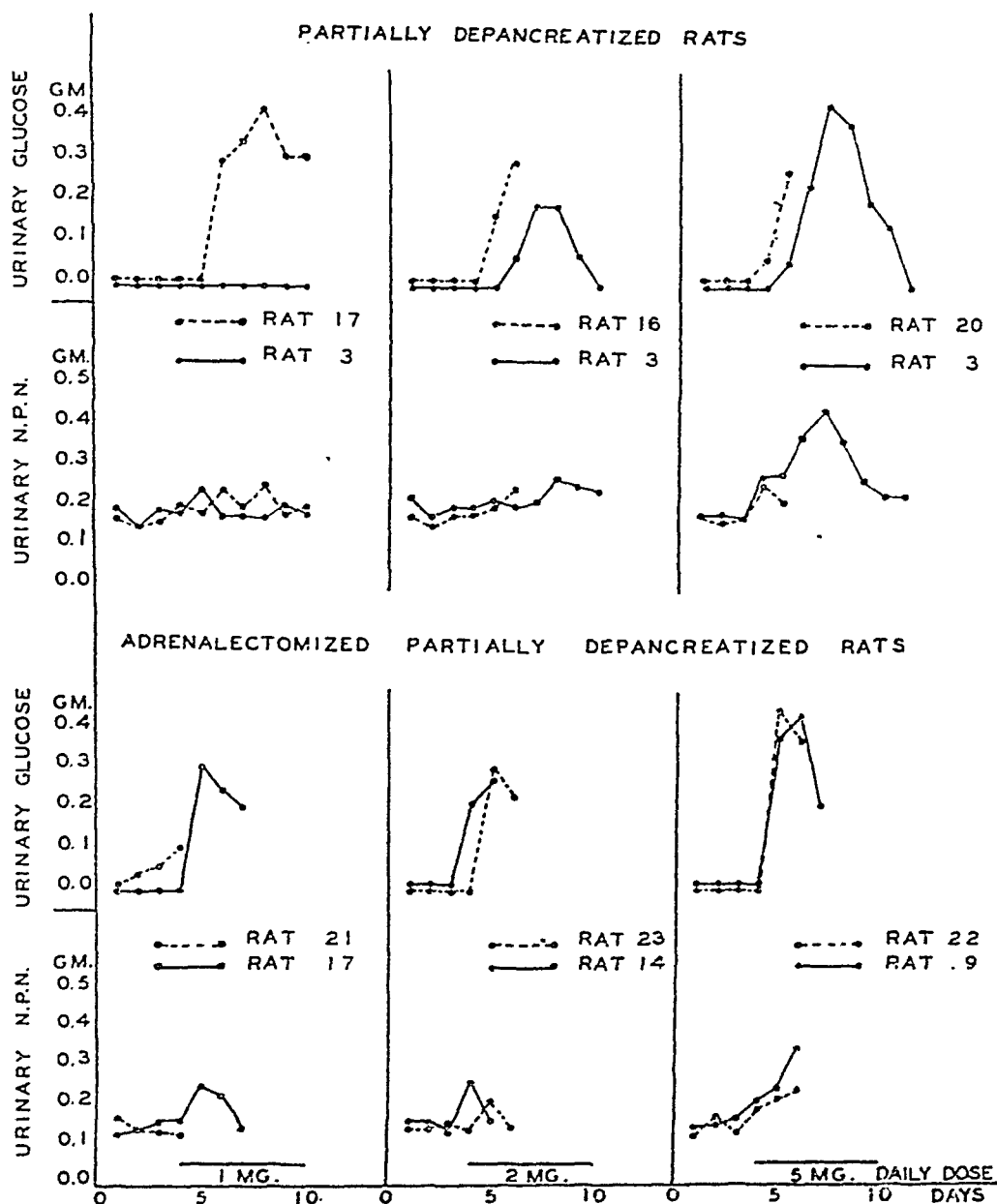
Fig. 2. The effect of 17-hydroxy-11-dehydrocorticosterone[2] on the excretion of glucose and non-protein nitrogen.

Renal excretion of sodium, chloride, potassium and inorganic phosphorus. Treatment with daily injections of 1, 2 or 5 mgm. of 11-desoxycorticosterone acetate was associated with a decrease in the renal excre-

[2] The treatment of these same animals with identical amounts of 11-desoxycorticosterone acetate failed to increase the excretion of glucose or non-protein nitrogen in every instance.

tion of sodium and chloride in all but one of the experimental animals (table 3). Equivalent quantities of 17-hydroxy-11-dehydrocorticoster-

TABLE 3

The effect of treatment with 11-desoxycorticosterone acetate or 17-hydroxy-11-dehydrocorticosterone on the renal excretion of sodium and chloride

ANIMAL NUMBER	HORMONE	RENAL EXCRETION			
		Na		Cl	
		Retention	Increased excretion	Retention	Increased excretion
Partially depancreatized					
A. 11-desoxycorticosterone acetate					
	mgm. per day	*per cent*	*per cent*	*per cent*	*per cent*
3	1	12		7	
17	1	7			5
3	2	11		9	
16	2	10		1	
3	5	7		0	
20	5	1		7	
B. 17-hydroxy-11-dehydrocorticosterone					
3	1	2		4	
17	1		1	4	
3	2	4		9	
16	2		19		14
3	5		14		10
20	5	20			12
Adrenalectomized, partially depancreatized					
A. 11-desoxycorticosterone acetate					
17	1		17	8	
21	1	34		13	
14	2	18		12	
23	2	20		8	
19	5	41		22	
22	5	21		4	
B. 17-hydroxy-11-dehydrocorticosterone					
17	1		6		7
21	1	30		53	
14	2	24		17	
23	2	20		20	
19	5		53		6
22	5		13	4	

one were not as effective in this respect. The excretion of potassium and inorganic phosphorus was studied in four partially depancreatized and

two adrenalectomized, partially depancreatized rats. In four of the six animals, there was an appreciable increase in the excretion of potassium during treatment with 11-desoxycorticosterone acetate. This compound had no consistent effect on the excretion of inorganic phosphorus. Treatment with 17-hydroxy-11-dehydrocorticosterone was followed by a much more striking increase in the excretion of inorganic phosphorus. (table 4). The marked increase in phosphorus and potassium excretion following treatment with 17-hydroxy-11-dehydrocorticosterone appeared to reflect the increased catabolism of protein which was observed during the period of treatment with this compound. Thus it appeared that treatment with 11-desoxycorticosterone acetate had a more striking effect on the regulation of sodium and chloride balance, whereas the effect of 17-hy-

TABLE 4

A comparison between the effect of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone treatment on the renal excretion of inorganic phosphorus and potassium in a typical case

(Rat 15—partially depancreatized)

PERIOD (4 DAYS)	RENAL EXCRETION	
	Inorganic phosphorus	Potassium
	mgm. per day	*m.eq. per day*
Control..	77	1.6
2 mgm. daily of 11-desoxycorticosterone acetate...	95	2.0
Control..	87	1.5
2 mgm. daily of 17-hydroxy-11-dehydrocorticosterone..	148	1.8

droxy-11-dehydrocorticosterone was more marked on phosphorus metabolism.

DISCUSSION. In preparing the experimental animals for this study, it was observed that adrenalectomy was followed by a complete disappearance of glycosuria in nine of ten partially depancreatized animals. These observations confirm the studies of Long and others (4).

Treatment with 11-desoxycorticosterone acetate was much more effective than 17-hydroxy-11-dehydrocorticosterone in inducing sodium and chloride retention. Injections of 11-desoxycorticosterone acetate were also followed by a small, but definite increase in potassium excretion without a significant increase in the excretion of inorganic phosphorus. It appears probable that this alteration in potassium excretion was a reflection of the direct effect of 11-desoxycorticosterone acetate on sodium: potassium balance (3). Wells and Kendall (7) have observed that treatment with 11-desoxycorticosterone and its acetate depressed the level of potassium and elevated the level of sodium in the blood serum to a

greater extent than did treatment with 17-hydroxy-11-dehydrocortico-sterone. It is probable that the glycosuria, ketosis and weight loss following treatment with 17-hydroxy-11-dehydrocorticosterone appreciably decreased the apparent sodium and chloride retaining effect of this compound. Treatment with 17-hydroxy-11-dehydrocorticosterone was much more effective than 11-desoxycorticosterone acetate in inducing glyco-suria, increased nitrogen excretion and ketosis. The striking increase in the excretion of inorganic phosphorus and non-protein nitrogen which accompanied the slight increase in potassium excretion during treatment with 17-hydroxy-11-dehydrocorticosterone acetate suggests that the abnormal excretion of these electrolytes reflected the effect of this compound in increasing gluconeogenesis.

It is of considerable interest to note that not all of the increased glucose excretion which was observed following 17-hydroxy-11-dehydrocorticos-terone therapy could be accounted for on the basis of increased glucose formation from protein. This observation was similar to the effect observed in phlorhizin-treated, adrenalectomized rats (6) and suggests that this substance has a direct inhibitory influence on glucose oxidation as well as on the formation of glucose from protein (6).

SUMMARY. Partially depancreatized and adrenalectomized, partially depancreatized rats were used to compare the physiological properties of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticoster-one. Treatment with 1, 2 or 5 mgm. of the latter compound was followed by glycosuria, ketonuria, an increase in the excretion of non-protein nitro-gen, potassium and inorganic phosphorus, loss in body weight and ulti-mately resulted in the death of the animal. However, all of the increased glucose excretion could not be accounted for on the basis of increased glucose formation from protein. Treatment with equivalent quantities of 11-desoxycorticosterone acetate failed to induce glycosuria, ketonuria or increased non-protein nitrogen excretion. A slight increase in potas-sium excretion, unaccompanied by an increased excretion of inorganic phosphorus, was noted following 11-desoxycorticosterone acetate therapy. Larger doses of 11-desoxycorticosterone acetate (10 mgm. daily) induced glycosuria in two of three animals so treated. In contrast to the effect of 17-hydroxy-11-dehydrocorticosterone, treatment with 1, 2 or 5 mgm. of 11-desoxycorticosterone acetate was followed by a striking decrease in the renal excretion of sodium and chloride in 11 of 12 experiments.

CONCLUSION

Striking differences in the principal physiological effects of 11-desoxy-corticosterone acetate and 17-hydroxy-11-dehydrocorticosterone were observed in partially depancreatized and in adrenalectomized, partially depancreatized rats.

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CONDITIONS IN WHICH THE LIVER RETAINS LACTIC ACID

H. BERRY IVY[1] AND LATHAN A. CRANDALL, JR.

From the Department of Physiology, University of Tennessee Medical School, Memphis

According to Cherry and Crandall (1), retention of lactic acid by the liver does not occur in normal unanesthetized dogs that are regularly fed. Himwich (2) has confirmed these observations using anesthetized dogs, and quotes the suggestion of Crandall that fasting may be an appropriate stimulus for retention.

Because previous observations have shown that lactic acid *may* be removed from the blood by the liver (3), and because McClure (4) has suggested that an observed increase in the pH of the blood passing through the liver may be explained by lactic acid retention, it has seemed desirable to investigate the conditions under which hepatic removal of lactic acid does occur.   Our studies have been carried out on normal, unanesthetized, angiostomized animals to avoid the disturbances of carbohydrate metabolism that may be produced by anesthesia and surgical procedures (1); it may be noted that previous investigations have involved indirect methods or acute experiments under anesthesia.

Methods: All experiments have been done on 12 to 15 kilo angiostomized dogs, 16 hours or more after the last meal.

"True" blood sugars were determined by Somogyi's modification of the Shaffer-Hartman method using Somogyi's second macro procedure for preparing blood filtrates.   Lactic acids have been determined by a modification of the method of Miller and Muntz (5).   Duplicate lactic acid analyses on twelve filtrates made from the same blood sample show a standard deviation from the mean of $\pm$ 0.8 mgm. per cent.   No change of less than two milligrams per cent has been considered significant.   The lactate and blood sugar values for the inflowing blood of the liver have been calculated on the basis that the hepatic artery supplies one-fourth of the total and the portal vein three-fourths.

RESULTS.   Four and twenty-five one hundredths grams of *dl*-lactic acid given orally to 4 dogs produced no significant increase in blood lactic acid level or in retention by the liver (table 1-A).   The control lactic acid levels in these animals are higher than in subsequent experiments, presumably

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[1] Abbott Laboratories Fellow in Physiology.

TABLE 1

Effect of lactic acid, sodium lactate and hydrochloric acid on hepatic retention of lactic acid

+ means output by liver, − means retention

EXPERIMENT	HEPATIC RETENTION, MGM. PER CENT			ARTERIAL LEVEL, MGM. PER CENT		
	Control	30 min.	60 min.	Control	30 min.	60 min.
A. 4.25 grams dl lactic acid in 200 ml. of water, orally						
112	+2	−1	−2	28	23	22
113	+6	+5	+6	23	18	21
114	+2	+6	+4	23	23	23
115	+2	+3	0	19	14	15
116	0	−2	0	13	16	17
Average ..	+2.4	+2.2	+1.6	21.2	18.8	19.6
B. 8.5 grams dl lactic acid in 200 ml. of water, orally						
127	+3	−3	0	11	28	22
128	+1	−3	0	8	16	11
129	+6	−1	−1	9	25	25
130	+4	−1	−1	9	15	17
Average ..	+3.5	−2.0	−0.5	9.3	21	18.8
C. 6.5 grams of sodium lactate (dl) in 200 ml. of water, orally						
106	−2	+1	+1	9	14	11
117	+5	+3	−1	17	17	22
118	0	−3	−3	13	22	27
119	+2	+2	−1	12	25	35
Average..	+1.3	+0.8	−1.0	12.8	19.5	23.8
D. 150 ml. 2.5 per cent Na lactate (dl) intravenously at 5.0 ml./min.						
110	+5	+2	−2	7	42	26
120	0 ·	+2	+1	11	26	18
120A	+1	+4	+2	16	27	22
E. 5.0 ml. of conc. comm. HCl in 200 ml. of water, orally						
105A	−1	0	+1	6	5	4
105B	−1	−1	−1	10	9	7
105C	+2	0	−1	14	12	9
F. 10.0 grams of NaHCO₃ in 200 ml. of water, orally						
102	+5	+2	+4	17	10	12
125	0	+1	0	8	53	43
126	−2	0	−1	5	6	6

because the dogs were led on chains from their cages to an adjoining room whereas in all later observations the animals were lifted from their cages and placed directly on a table.

The blood glucose showed no significant changes in these experiments. Since this is also true of most of the subsequent experiments, only noteworthy changes will be mentioned although analyses for glucose were made on every blood sample.

When the oral dosage of lactic acid was increased to 8.5 grams (table 1-B) in four dogs, the arterial level doubled, and the previous slight hepatic output of lactic acid changed to a slight retention. After 6.5 grams of sodium lactate (table 1-C) the arterial lactic acid level increased more slowly but doubled at 1 hour, and in two of the four animals there was a change toward hepatic retention that is suggestive. However, a comparable increase in blood lactate produced by the intravenous injection of 3.75 grams of sodium salt over a period of 30 minutes (table 1-D) resulted in a tendency for the liver to retain lactic acid in but one of three experiments. In general it may be said that the effect on hepatic activity of doubling the blood lactic acid is certainly not striking, and leads to no such degree of retention as is uniformly observed in fasted animals that have much lower blood lactic acid concentrations (see below). It is also evident that moderate increases in the blood lactic acid level are not necessarily associated with retention of lactic acid by the liver.

Hydrochloric acid and sodium bicarbonate were given orally in an attempt to test McClure's theory. The acid produced a suggestive decrease in blood lactic acid level but no evidence of increased hepatic retention (table 1-E). Sodium bicarbonate had no demonstrable effect on either (table 1-F).

The effect of carbohydrate deprivation was studied in 6 dogs which were fasted for periods up to 7 days or were maintained for longer periods on nothing but 100 ml. of olive oil per day. There was consistently a hepatic removal of lactic acid from the blood in these animals (table 2). In each instance the fast or fat feeding period was terminated by the administration of 20 grams of glucose, and as shown in table 2 this did not reduce the amount of lactic acid removed by the liver per unit volume of blood within the first hour after glucose. The blood lactic acid level rose after glucose administration.

As shown in table 3, the intravenous injection of physiological amounts of epinephrine for a 10 minute period roughly tripled the blood lactic acid level, and only a slight decline occurred during the next 20 minutes. In the six dogs that had had their last meal within 20 hours, epinephrine produced no demonstrable change in retention of lactic acid by the liver. This again indicates that an increase in the blood lactic acid level is not necessarily associated with hepatic retention. Dogs that had fasted 36

TABLE 2

Hepatic retention of lactic acid in starvation and following termination of fast with glucose

+ indicates output by liver, — indicates retention

EXPERI-MENT	HEPATIC RETENTION, MGM. PER CENT								ARTERIAL LACTIC ACID, MGM. PER CENT							
	Hours of starvation															
	24	48	72	120	168	216	240	288	24	48	72	120	168	216	240	288
121A	−3	−4	−4	−4	−2				8	9	7	9	8			
121B	−2	−1	−4	−6	−3				11	15	12	11	12			
122*		−4		−1	−4	−3	−6	−3	11			10	8	9	15	5

20 grams of glucose after control sample taken

EXPERIMENT	HOURS STARVATION	CONTROL	30 MIN.	60 MIN.	CONTROL	30 MIN.	60 MIN.
121A	168	−2	−3	0	8	22	29
121B	168	−2	−1	−4	11	15	12
122	288*	−3	−4	−3	5	7	9
123A	408*	−7	−6	−6	12	17	23
123B	432*	−4	−6	−4	8	11	11
124	480*	−2	−2	−3	8	8	10

* One hundred milliliters of olive oil per day for varying periods of fast.

TABLE 3

Hepatic retention (or output) of lactic acid following intravenous injection of epinephrine in post-absorptive and starving dogs

Injection period 10 min.; rate 0.004 mgm. epinephrine/kilo/min.   + indicates output, — retention.

EXPERIMENT	HOURS SINCE FOOD	HEPATIC LACTIC ACID RETENTION, MGM. PER CENT			ARTERIAL LACTIC ACID CONCENTRATION, MGM. PER CENT		
		Control	10 min.	30 min.	Control	10 min.	30 min.
143	16–20	+2	−1	+1	11	39	26
144	16–20	−3	+2	+2	8	28	24
145	16–20	+1	+1	+1	10	31	30
146	16–20	+1	−1	0	17	31	29
147	16–20	−1	−1	−1	8	29	33
148	16–20	0	−2	−2	8	17	16
Average		0.0	−0.33	+0.16	10.3	29.2	26.3
140	48	−2	−6	−2	9	24	22
141	36	−2	−6	−4	10	33	24
142	48	−2	−7	+1	9	40	23
149	48	−2	−3	+3	6	26	28
150	72	−1	−4	−2	6	21	25
151	72	−3	−8	−10	9	20	25
Average		−2.0	−5.7	−2.3	8.2	27.2	24.5

to 72 hours, and were with one exception showing significant hepatic reten-
tion of lactic acid, responded to epinephrine in all but one instance with
definite increases in lactic acid retention by the liver when the epinephrine
injection was terminated (10 min. period).   Twenty minutes later (30 min.
period) 4 of the dogs had returned to the pre-injection rate of removal.
The fact that epinephrine increases the amount of lactic acid retained per
unit volume of blood does not necessarily indicate, in the absence of blood
flow determinations, an increased retention per unit time.   However it
should be pointed out that it is necessary to postulate an average decrease
in blood flow to 35 per cent of its original value if one would explain the
observed changes at the 10 minute period on the basis of blood flow varia-
tion.   The hepatic output of glucose was considerably increased at the
10 minute period in most experiments, and the blood sugar level more than
doubled.   At the 30 minute period (20 min. after stopping the epinephrine
injection) hepatic glucose output had either ceased or actual retention was
occurring in 8 of the 12 experiments, and the blood sugar level had fallen
sharply.

TABLE 4

*Comparison of average hepatic retention of lactic acid in post-absorptive state
and during fasting*

	NUMBER OF DETERMINATIONS	AVERAGE RETENTION	STANDARD ERROR
		mgm. per cent	
Post-absorptive.....................	31	−0.35	±0.51
Starvation........................	20	−3.6	±0.93

DISCUSSION.   Our data support previous evidence (1, 2) that lactic acid
is not retained by the liver when the animal is in the post-absorptive state.
The mean of 31 observations on our dogs fed not more than 24 hours pre-
viously shows a retention of 0.35 mgm. per 100 ml. of blood, which is less
than its standard error of ± 0.51.

On the other hand, the liver consistently does retain lactic acid in fasted
animals.   The mean of 20 observations on dogs 24 hours or more after the
last meal shows a retention of 3.6 mgm., with a standard error of ± 0.93.
Fasting is the only procedure we have used that leads to such unequivocal
results.   In post-absorptive animals, doubling or tripling the blood lactate
level by the oral or intravenous administration of sodium lactate, or the
oral administration of lactic acid, or by intravenous injections of epi-
nephrine, does not produce a clearly significant change in the behavior of
the liver.   A suggestive trend toward retention occurs when lactic acid is
given orally in the larger doses.

We believe that previous evidence, supported by the data here presented,
shows that hepatic lactic acid retention and conversion to glucose is not a

mechanism for maintaining acid base balance in non-fasting animals. In fact, we have been unable to obtain any definite results that can be interpreted as supporting the hypothesis of McClure (4), even when a mineral acid was given by mouth to the limit·of tolerance. Our observations do not invalidate McClure's theory, but suggest that further evidence should be presented if it is to be accepted even for the condition of acidosis.

Since we do not know the effect on hepatic blood flow of the amount of epinephrine we injected, it is impossible to interpret these data in terms of hepatic lactic acid·retention per unit time. It is evident that epinephrine does not significantly affect the behavior of the liver toward lactic acid in the non-fasting animal; however the increased retention per unit volume following injections of epinephrine in the fasting dog suggests that when liver glycogen reserves are low, epinephrine may bring about increased gluconeogenesis.

It has been shown previously (6) that the metabolism of fat is qualitatively different in the post-absorptive and fasting states. Lactic acid retention (and presumably gluconeogenesis from lactic acid) has now been shown not to occur in the regularly fed animal and to be present during fasting or fat feeding. It may therefore be regarded as another aspect of the special metabolism of the glucoprivic state.

SUMMARY

The non-occurrence of hepatic lactic acid retention in the non-fasting animal is confirmed. Significant lactic acid retention is present during fasting or fat feeding. The administration of lactic acid or sodium lactate by mouth, or the intravenous administration of sodium lactate or epinephrine, do not produce clearly significant lactic acid retention by the liver. Large amounts of lactic acid orally, however, result in a suggestive trend toward retention. Oral administration of acid or base does not affect lactic acid retention by the liver as estimated by our methods.

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RESPIRATORY AND METABOLIC EFFECTS OF HYPOTHERMIA

D. B. DILL and W. H. FORBES

With the technical assistance of FRANK CONSOLAZIO

From the Fatigue Laboratory, Harvard University, Morgan Hall, Boston, Massachusetts

Recent developments have revived the interest of physiologists in the effects of cold on homiothermic animals. The monograph of Benedict and Lee (1) on hibernation gives us a clear picture of the metabolic, circulatory and respiratory responses of the marmot as cold brings about the state of hibernation. Smith and Fay (2) in treating cancer have induced experimentally for the first time in man body temperatures as low as 24°C. Experiences of troops in Finland, Norway and Greece and of our own armed forces in the Arctic and in high altitudes have emphasized the need for a clear insight into such questions as adaptation to, and protection against, cold and the nature of the breakdown resulting from prolonged exposure to cold. These questions call for knowledge of metabolic, respiratory and cardiovascular responses to cold, of the relation of fatigue and of food to the ability to withstand cold, and of the protective characteristics of various types of shoes and clothing.

As an outgrowth of the treatment of cancer patients developed by Smith and Fay, an experimental hypothermic treatment of schizophrenic patients has been instituted at the McLean Hospital in Waverley, Massachusetts. The rationale of the treatment, the protocols of the cases and the therapeutic results will be reported elsewhere by Drs. Kenneth J. Tillotson and John H. Talbott (3). Briefly, the naked and lightly anesthetized patient is placed between rubberized blankets. These contain rubber coils through which a refrigerated fluid circulates. It enters at a temperature of −2 to −5° during the induction period, and since heat transfer through rubber is moderately effective the skin temperature is lowered by 20°C. or more. Peripheral vasoconstriction and shivering occasionally delay the fall in rectal temperature for some hours. When it does begin to fall, regulation is effected by adjusting the flow and temperature of the circulating fluid. Within 1 to 4 hours the administered anesthetic has been dissipated; thereafter cold itself provides the only anesthesia. We have been able to make many physiological observations on these patients during treatment and are reporting here metabolic and respiratory responses.

TABLE 1

Metabolic changes in hypothermia

SUBJECT	HEIGHT	WEIGHT	AGE	HOUR OF TREATMENT	RECTAL TEMP.	RESPIRATORY VOLUME STP	rq	CO_2 OUTPUT	O_2 USED	ESTIMATED NORMAL BASAL O_2 CONSUMPTION	OBSERVED MR/ESTIMATED BASAL MR	REMARKS
	cm.	kgm.	yrs.		°C.	l./min.		cc./min.	cc./min.	cc./min.		
Mrs. H. M.	165	59.0	39	3	37.2	6.3	0.92	241	262	208	1.26	Shivering
				13	25.5	9.6	0.68	223	328		1.58	
				21	29.4	16.3	0.78	343	443		2.13	Shivering
Miss S. E.	168	47.6	30	2	37.0	9.4	0.79	205	259	195	1.33	
				4	35.0	23.9	0.81	564	700		3.59	Quiet
				10	30.0	12.6	0.78	281	358		1.84	Quiet
				24	26.7	7.8	0.73	111	153		0.78	
				29	28.3	7.0	0.72	93	132		0.66	
Mr. W. D.	178	73.5	46	0	37.0	10.9	0.86	220	256	255	1.00	Before
				8	34.4	28.3	0.87	580	750		2.94	Struggling
				24	28.0	14.0	0.78	256	330		1.29	Quiet
				33	29.4	18.1	0.73	235	324		1.27	Restless
				48	31.4	16.1	0.51	195	386		1.51	Active
Miss S. J.	166	55.4	23	0	37.2	8.4	0.79	197	248	208	1.19	Before
				9	35.6	16.6	0.68	437	647		3.11	
Miss B. E.	175	67.6	25	10	34.3	14.1	0.74	392	530	232	2.28	
				24	32.8	16.3	0.75	415	556		2.40	
Mr. S. R.	177	59.4	16	1	38.3	6.0	0.69	147	214	266	0.80	Restless
				9	36.1	18.5	0.81	617	760		2.86	Struggling
				23	35.0	14.9	0.72	407	570		2.14	
Miss H. I.	163	58.1	37	8	33.0	13.4	0.79	302	383	207	1.85	
				28	32.3	4.8	0.79	87	112		0.54	
Miss P. M.	160	49.9	38	1	37.6	8.9	0.78	219	280	189	1.48	
				11	30.0	9.8	0.73	174	237		1.25	
				28	31.1	6.9	0.73	113	155		0.82	Quiet
Miss M. F.	161	54.0	25	0	38.0	6.8	1.00	160	160	199	0.80	Resting
				23	32.2	12.3	0.74	267	363		1.84	
				32	39.9	10.4	0.80	206	256		1.28	

RESULTS. The metabolic measurements are shown in table 1. Since it was usually impossible because of the disturbed mental state to determine the basal metabolic rate before induction of hypothermia unless the

patient was anesthetized, we have incorporated in this table the records of height, weight, age and sex and then calculated from the Aub-DuBois standards the basal oxygen requirement. The observed oxygen consumption was then expressed both as cubic centimeters per minute and as a ratio, taking the estimated basal metabolic rate, BMR, as unity. Under the experimental conditions there were five instances where the observed oxygen consumption fell below the predicted basal level. In 17 instances the temperature was below 37° and yet the metabolic rate was elevated. It was commonly more than doubled in the early stages of induction, and in some instances was increased 50 per cent even when the temperature was as low as 30°. This elevation in metabolism that persists despite the falling temperature is chiefly a consequence of involuntary shivering and of voluntary muscular activity. While both are suppressed in deep

TABLE 2

Typical notes on muscle rigidity

SUBJECT	TEMPERA-TURE	NOTES
	°C.	
Mrs. H. M........	30.0	Restless and rigid
Miss S. E........	26.7	Body rigid
Mr. W. D........	31.1	Rigid and resistive
Miss S. J.........	29.5	Short periods of restlessness and rigidity
Miss B. E........	33.1	Much quieter, relaxed
Mr. S. R.........	34.8	Extremities very rigid
Miss H. I........	37.9*	Body very rigid
Miss P. M........	30.6	Limbs rigid and resistive
Miss M. F........	30.6	Abdomen board-like; body, particularly limbs, rigid

* Induction period.

anesthesia, experience indicates that hypothermia can be maintained with greater safety if cold alone is relied upon for anesthesia. While shivering may persist even at 30°, the attacks become less frequent and are usually less intense below this temperature. Voluntary movements, sometimes requiring restraint, continue at 30° and even below. Our metabolic measurements probably do not reflect the greatest periods of activity, for then it is impossible to keep the face mask in place.

In addition to the voluntary movements and the shivering there was another type of muscular contraction which probably increased the metabolism a small amount. In every patient exposed to low temperatures some flexor muscles at times assumed a state of contraction. It was often difficult to straighten the arm enough to draw blood from the antecubital vein, and the protocols contain many notes such as those quoted in table 2. This state can hardly be one of voluntary contraction, since the performance of so much static work would bring on exhaustion reflected by a degree

of hyperpnea and lactic acid accumulation surpassing those seen in any of these patients. Neither is it properly described as "cold rigor," for in isolated muscle the lower the temperature, the slower the development of rigor, as shown by Baumann (4). It does not depend simply on body temperature, since it may be present when the temperature is only slightly reduced and absent at low temperatures. Its mechanism remains to be elucidated.

The respiratory quotients (table 1) must be viewed with some reserve since the patients were not always in a steady state preceding and during the period of measurement. Although a considerable amount of glucose solution was given by stomach tube (usually 10 grams per hour), there was a tendency toward a decreased utilization of carbohydrate. This was seen in six out of seven patients on whom an observation was made early in the treatment. This trend, assuming its reality, may depend on depleted reserve of carbohydrate or, on the other hand, on inability to use it. In this connection it is reported (5) that an effective means of rendering the dog's liver glycogen-free is induction of violent shivering by extreme cold. This suggests that early depletion of carbohydrate rather than incapacity for its utilization is involved. There is a significant additional fact: acetonuria may be intense. This implies that there is no unusual capacity for utilizing body fat without ketosis by man within the range of temperatures studied.

The respiratory volume in most patients is large in comparison with the volume of oxygen removed. Under conditions of rest normal persons remove from 3 to 5 per cent of oxygen from inspired air, and this proportion does not change much in moderate activity. There is a tendency in these patients for this proportion to be reduced as the temperature falls. When the lower temperatures are reached, less than 2 per cent oxygen may be removed and the expired air may contain as little as 1.5 per cent CO_2. The significance of this alteration in respiratory regulation will be discussed in connection with observations on the properties of arterial blood.

Respiratory function of the blood. The changes to be expected in properties of the blood include, on the one hand, those that can be predicted from our knowledge of the physicochemical properties of the system and, on the other hand, those that depend on the physiological reactions to cold. In the first category are the changes in solubility of gases, the decrease in base-binding capacity of proteins and the increase in affinity of hemoglobin for oxygen. In the other category one may find a decrease in available base because certain anions accumulate, a change in hemoglobin concentration because of a redistribution of body fluids, and responses of pCO_2 and pH that are dependent on the foregoing factors as well as on the effect of cold per se on tissues involved in respiratory regulation.

The effect of temperature on the base-binding capacity of plasma proteins can be calculated from existing data. The equation at 38°, according to Van Slyke, Hastings, Hiller and Sendroy (6), is

$$BP_s = 0.104 \; (P)_s \; (pH_s - 5.08) \tag{1}$$

where BP_s = milliequivalents of base bound per liter of plasma
    P_s = grams of protein per liter of plasma
   0.104 = a factor measuring buffer value
   5.08 = the pH of minimal base binding at 38°

The pH of minimal base binding, commonly referred to as pI, varies with temperature. The approximate relation for plasma protein, according to Stadie, Austin and Robinson (7) is

$$\frac{\Delta pI}{\Delta t} = -0.02$$
$$\text{or } \Delta pI = -0.02 \; \Delta t$$

By substitution, the general equation taking temperature into account is

$$BP_s = 0.104 \; P_s \; [pH_s - (5.08 + \Delta pI)]$$
$$\text{or } BP_s = 0.104 \; P_s \; [pH_s - (5.08 - 0.02 \; (t_0 - 38°))] \tag{2}$$

where t_0 = observed temperature in °C.

Equation (2) has been used for calculating proteinate of serum in these experiments; these results are being published elsewhere.

In our studies of acid-base balance in the blood the CO_2 and O_2 contents are determined directly on the Van Slyke apparatus. Similar analyses are made of blood equilibrated at 37° and at a CO_2 tension of about 40 mm.Hg. In most instances, when the body temperature is reduced a second portion is equilibrated at body temperature; the CO_2 content of this gives the CO_2-combining capacity of the blood *in vivo*. With suitable correction for dissolved oxygen, one should obtain the same O_2 combining capacity in blood samples equilibrated at different temperatures, but the CO_2-combining capacity increases as the temperature decreases. This depends on the decreasing base-binding capacity of blood proteins—both of the plasma proteins, as described above, and of hemoglobin as well.

In view of the heterogeneity of the system, the effect of cold on the base-binding capacity of blood is too complicated to be described precisely in simple terms. An empirical relation has been worked out that holds with sufficient accuracy for such experiments as these. It is based both on such pairs of observations as those just described and also on two experiments in which normal blood was equilibrated with CO_2 tensions ranging from 10 to 80 mm. and at temperatures of 23, 30 and 37°. The empirical relation between T_{40} (the millimols of CO_2 in oxygenated

blood at a CO_2 tension of 40 mm.Hg), and the change of T_{40} per degree, is as follows:

T_{40} at 37°, mM	$\Delta T_{40}/\Delta t$
22	−0.45
20	−0.37
18	−0.29
16	−0.24
14	−0.21
12	−0.19
10	−0.17

Values for pH and for pCO_2 in samples of blood so analyzed and equilibrated can be calculated in the usual way, remembering the effects of temperature on pK′ and on the solubility of CO_2. The former relation, according to Cullen, Keeler and Robinson (8), is

$$\frac{\Delta pK'}{\Delta t} = -0.005$$

The solubility of CO_2 in serum, taking the value for 38° established by Van Slyke, Sendroy, Hastings and Neill (9) as a standard, can be calculated for other temperatures assuming that the effect of temperature on solubility is the same as in water and that the water content of serum is 940 cc. per liter. The calculated values are given in the following table. Factors for oxygen solubility in blood of normal hemoglobin concentration are given in the same table. These are based on the factor for normal blood at 38° established by Sendroy, Dillon and Van Slyke (10) and are calculated to other temperatures on the assumption that the solubility varies with temperature as in pure water.

TEMPERATURE	CO_2	O_2
°C.	$mM/l.$ serum	$mM/l.$ blood
25	0.0424 pCO_2	0.00163 pCO_2
26	0.0411	0.00161
27	0.0399	0.00158
28	0.0388	0.00155
29	0.0379	0.00153
30	0.0370	0.00150
31	0.0361	0.00148
32	0.0353	0.00146
33	0.0345	0.00144
34	0.0337	0.00143
35	0.0329	0.00141
36	0.0321	0.00139
37	0.0313	0.00138
38	0.0306	0.00136
39	0.0299	0.00135
40	0.0293	0.00133

Temperature affects both the base-binding capacity of hemoglobin and also, as described experimentally by Brown and Hill (11), its combination with oxygen.   For the form of the curve at constant pH we have the data given by Dill, Edwards, Florkin and Campbell (12).   They also give the change in position with pH_c.   It is somewhat more convenient to have their results in terms of pH_s: this calculation we have made, using the relation between pH_s and pH_c given in an alignment chart by Dill, Edwards and Consolazio (13).

STANDARD CURVES AT pH = 7.4

%HbO2	pO2 23°	30°	37°
2	1.2	1.6	2.1
4	2.1	2.8	3.8
6	3.0	4.1	5.5
10	4.5	6.1	8.2
15	6.0	8.1	10.9
20	7.3	10.0	13.4
30	9.8	13.3	17.9
40	12.0	16.4	22.0
50	14.4	19.6	26.3
60	17.0	23.1	31.1
70	19.8	26.9	36.1
80	25.0	34.1	45.7
85	28.3	38.5	51.7
90	33.6	45.7	61.4
94	41.0	55.9	75.0
96	48.0	65.3	87.7
98	61.8	84.2	113.0
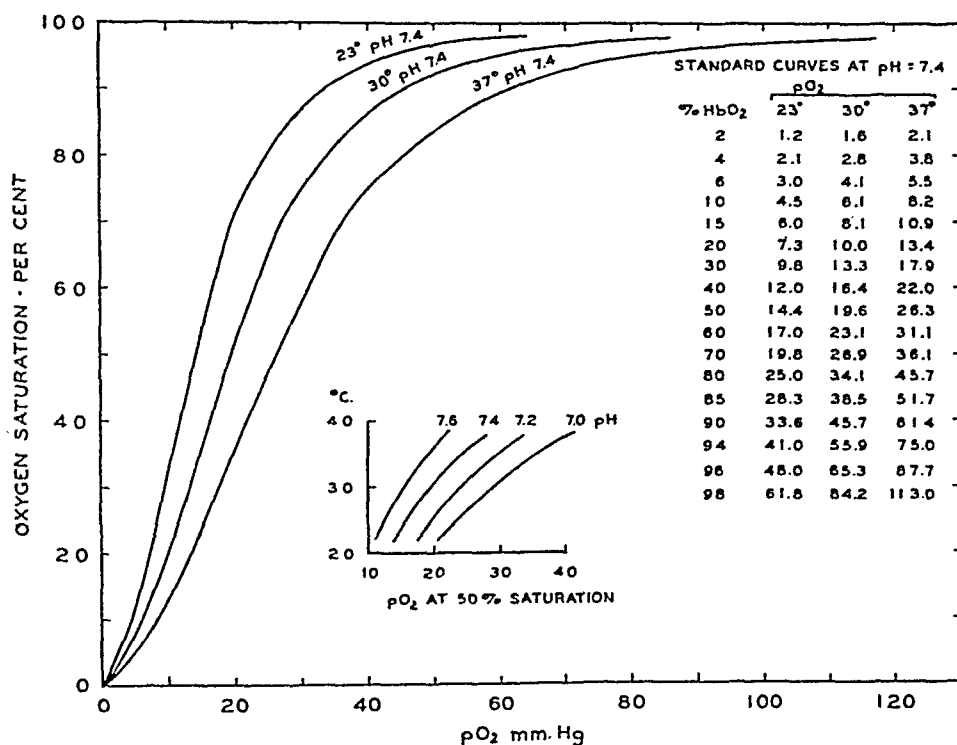
Fig. 1. Oxygen dissociation curves of human blood at a pH_s of 7.4 and at 23, 30 and 37°C.   The inserted figure shows the relation of pO_2 at one-half saturation to temperature at four pH values.   Since oxygen dissociation curves can be transformed from one position to another by multiplying abscissae by a constant factor, it is possible to construct a dissociation curve for any condition within the given limits of pH and temperature.

The results of the foregoing calculations yield the curves of figure 1. The three curves of figure 1 A define the combination of oxygen with hemoglobin at 23°, 30° and 37°C.   Figure 1 B gives the relation between the position of the curves (pO_2 at one-half saturation) and four pH values within the same temperature range.   Assuming that the form of the oxygen dissociation curve is independent of pH and temperature, these figures yield by interpolation the percentage saturation for any pO_2 and any pH_s if varied by CO_2 within the given limits or, if desired, a complete oxygen dissociation curve at any desired condition.

TABLE 3.

Respiratory properties of the blood

SUBJECT	HOUR OF TREAT-MENT	BODY TEM-PERA-TURE	HbO2 CAPACITY	HbO2 CONTENT	TOTAL CO2	pH	pCO2	T40 At body tempera-ture	At 37°
		°C.	mM/l.	per cent	mM/l.		mm. Hg	mM/l.	mM/l.
Mrs. H. M.	0	37.0	7.8	96.8	22.6	7.41	40.4	22.4	22.4
	8	30.0	9.5	99.2	15.4	7.37	28.8	17.8	16.1
Miss S. E.	0	37.3	7.4	99.1	23.3	7.35	48.0	21.7	21.8
	4	34.6	9.4	93.9	20.6	7.28	47.5	19.0	18.3
	20	24.2	10.8	93.0	20.0	7.22	45.2	19.0	16.0
	29	29.5	9.3	97.2	17.3	7.33	34.0	18.6	16.7
Mr. W. D.	0	37.0	8.8	100.0	20.8	7.37	41.0	20.4	20.4
	7	35.0	9.4	98.8	17.0	7.31	37.8	17.3	16.8
	21	26.7	9.9	100.0	15.1	7.38	26.7	18.3	15.8
	45	29.5	10.4	93.8	7.8	7.22	19.5	11.9	10.7
Miss S. J.	0	37.0	8.9	96.2	19.0	7.43	33.5	20.3	20.3
	9	35.1	9.0	96.0	19.1	7.33	40.4	19.0	18.4
	25	26.9	9.5	97.7	17.0	7.38	30.0	19.1	16.6
Miss B. E.	10	34.1	8.1	93.7	21.1	7.31	44.2	20.1	19.1
	21	31.6	8.1	96.7	17.9	7.41	30.0	20.0	18.3
	29	33.4	7.1	97.2	19.0	7.35	37.0	19.4	18.3
Mr. S. R.	0	37.0	9.0	95.5	21.7	7.39	41.8	21.2	21.2
	9	36.3	9.7	95.7	20.5	7.26	51.5	18.3	18.1
	22	36.1	10.0	97.3	15.3	7.23	41.2	14.9	14.7
	31	38.5	9.2	95.7	16.3	7.21	44.0	15.5	15.8
Miss H. I.	9	32.2	8.2	92.4	18.5	7.28	40.6	18.3	17.0
	21	31.2	7.7	92.1	18.9	7.33	36.2	19.6	17.9
	38	38.3	6.6	95.6	19.6	7.24	49.8	18.0	18.3
Miss P. M.	0	37.0	6.9	95.7	22.0	7.38	41.2	21.5	21.5
	21	32.1	7.1	100.0	19.1	7.38	33.5	20.2	18.6
	34	37.7	6.4	96.0	16.7	7.35	33.8	17.8	18.0
Miss M. F.	0	37.0	7.5	97.2	21.1	7.39	39.2	21.1	21.1
	23	33.0	9.2	96.0	16.4	7.33	33.8	17.6	16.6
	32	40.0	6.8	91.5	17.6	7.33	51.8	15.1	15.8

A simple application of the foregoing principles has been made. The observations on percentage saturation (table 3) have been segregated into three temperature ranges: 35° or above, 30° or below, and intermediate

values. The averages, together with the estimated pO_2 values, are as follows:

NUMBER OF CASES	MEAN TEMPERATURE	MEAN SATURATION	MEAN pH$_s$	pO_2
	°C.	per cent		mm. Hg
15	37.1	96.3	7.35	98
8	32.8	95.3	7.33	74
6	27.8	96.8	7.33	70

While we have no analyses of alveolar air, the fact that the ventilation was increased out of proportion to the increase in oxygen consumption implies that the alveolar pO_2 was greater than usual. Despite hyperventilation, the pO_2 of arterial blood declines with decreasing body temperature. This is not what one might predict, but there are several factors which may have contributed to this result. The slowing-down of the various chemical reactions in the blood which are concerned with the uptake of oxygen in the lungs may be a contributing factor, but it is probable that reduction in the rate of diffusion through the lung membrane is the most important cause. In one patient edema was evident, and it may be that a slight accumulation of fluid in the alveoli may occur at subnormal body temperatures.

It is notable that almost without exception the pH is on the acid side and the CO_2-combining capacity, even when measured at body temperature, is reduced. The state is one of acidosis partially but not wholly compensated for by hyperventilation. One patient (Mr. W. C.) experienced about twice as great a reduction in CO_2-combining capacity as any other and was also one of the most acidotic. He was also one of the most combative.

While the acid-base balance of the blood probably is chiefly responsible for the respiratory regulation in these patients, it is possible that the oxygen tension in arterial blood is reduced enough to stimulate respiration through the mediation of the carotid body. Reference may be made to the recent work of Asmussen and Chiodi (14) describing the rôle of the arterial pO_2 in regulating respiration.

The distribution of body water and the exchange of fluids will be discussed elsewhere and are mentioned here because of the considerable fluctuations observed in serum protein concentration and in the proportion of hemoglobin. In the early experiments little fluid was supplied and the hemoglobin increased, in the case of Miss S. E. by one-half. In most of the later cases the hemoglobin remained more nearly normal, but it decreased by one-third in Miss H. I.

DISCUSSION. Our observations on the metabolic rate in hypothermia

do not agree well with those reported by Smith and Fay (2). Using an indirect method, they commonly found a reduction ranging from −6 to −25. Our results are what one would expect from the numerous measurements made by Benedict and Lee (1) on the marmot as it enters hibernation. Only when shivering moderates should one expect a reduction in metabolic rate. It is interesting in this connection to speculate on the degree to which processes of homeostasis are thrown off balance when the body temperature is so greatly reduced. While shivering may demand more than twice the basal energy exchange, chemical processes, notably those of an enzymatic nature, are greatly slowed. This field deserves further exploration.

It is of interest to collate our observations on man at low body temperatures with those described in Walther's classic *Beiträge zur Lehre von der thierschen Wärme* (15). Walther observed that in rabbits killed while at a temperature of 20° the lungs were filled with blood and a watery serous exudate extended through the parenchyma and bronchioles. This fits in with our observation that the pO_2 in arterial blood is reduced: conditions for diffusion may be unfavorable because of pulmonary edema.

Another observation of Walther's may be interpreted similarly. While rabbits at 20° do not shiver and will die if left in a cool place, they can be revived, with slowly rising body temperature, by means of artificial ventilation, even though the air temperature may be 10 to 12°. (Walther claimed that this was the first proof that animal heat depends on the entrance of air into the lungs.) It may be that the arterial oxygen pressure is so greatly reduced by pulmonary edema that vital processes, including shivering, sink to lower and lower levels until they are terminated. Artificial ventilation raises the oxygen partial pressure enough to swing the balance in the other direction. In this connection Nielsen and others (16) have shown that shivering is inhibited by anoxia, even at ordinary body temperatures. Since it is also reduced in intensity by low temperature, it seems likely that a combination of cold and anoxia may produce a complete cessation of shivering and in effect a poikilothermous animal. An additional possibility is that the alkalosis of artificial ventilation may contribute to the result that so surprised Walther.

In general, any state that handicaps oxidative processes in the nervous system inhibits shivering. Besides cold, anesthesia and anoxia, mention should be made of the discovery by Dworkin and Finney (17) that shivering is inhibited in insulin hypoglycemia. Possibly in our experiments shivering abates not entirely because of falling temperature but in part because depletion of carbohydrate reserves lowers the blood sugar significantly. This question is being investigated.

Walther was the first to point out that cold produces a state of anesthesia

in which animals are rendered suitable for operative procedures. Muscles remain irritable while the reduction in peripheral blood flow lessens hemorrhage. Parker (18) has commented on this phenomenon.

Numerous observations like those of Walther prove that most mammals are apt to die if their temperature falls below 20° and almost certain to die before 10° is reached. Yet hibernating animals can survive a temperature of 2 or 3°C. Does this imply the possession of body constituents of unusual physicochemical properties? So far as we can find, Tait was the first to suggest that their lipoids may have unusually low solidification

TABLE 4

Solidifying points of body fats

SPECIES	AUTHOR	SOLIDIFYING TEMPERATURE
		°C.
A. Hibernating mammals		
Hedgehog (*Erinaceus europaeus*).....	Pawletta (20)	+3 to +5
Marmot (*Arctomys sp*).............	Pritzker and Jungkunz (21)	−15
Skunk (*Mephitis sp*)................	Pawletta (20)	+24
Bear (*Ursus torquatus*)............	Ueno and Kuzei (22)	30*
B. Nonhibernating mammals		
Badger (*Meles taxus*)...............	Lewkowitsch (23)	17–19
Hare (*Lepus timidus*)...............	Lewkowitsch (23)	17–30
Rabbit (*Lepus cuniculus*)...........	Lewkowitsch (23)	17–30
Sheep.............................	Lewkowitsch (23)	36–41
Ox................................	Lewkowitsch (23)	35–37
Dog...............................	Lewkowitsch (23)	21–23
Cat...............................	Lewkowitsch (23)	24–26
Hog...............................	Lewkowitsch (23)	27–30
Man...............................	Lewkowitsch (23)	15

* Melting point.

temperatures (19). This hypothesis was proposed after he had demonstrated that, while the excised heart of a hibernating animal will continue to beat at 0°, that of nonhibernating animals ceases to beat at 17°.

Data presented in table 4 bear out Tait's suggestion. The two animals on which observations have been made and which are known to hibernate throughout the winter have fats that remain fluid at low temperatures. The skunk and the bear are often active in midwinter and are therefore in a special category. Furthermore, the recorded solidifying temperature of skunk fat, 24°, does not correspond to the observation (24) that much of the fat is liquid at ordinary temperatures, separating into two phases as it is cooled. The data in table 4 must be considered as approximations, since

the solidification temperature cannot be determined precisely and certainly varies with diet, if not also with season. The characteristics of fats from different parts of the body are also known to vary.

The picture of an inadequately clothed man lost in a blizzard is illuminated by our findings. So long as he continues to walk, production of heat may balance its loss. Eventually exhaustion not only forces him to stop but may also, mediated by low blood sugar, keep shivering at a low level. As his temperature falls, shivering becomes less intense and eventually coma and death ensue.

Finally, we may say that hypothermia is a state of light anesthesia in which certain functions, such as those concerned with temperature regulation and acid-base balance, remain moderately effective. While the total metabolic exchange is apt to be above the basal level, this does not exclude the possibility that such organs as the brain, the liver and the kidney decline in activity with decreasing temperature. Studies are being continued in this direction.

SUMMARY

The total energy exchange throughout long periods of subnormal body temperature may remain above the basal level and may be two or even three times the basal level. This depends on shivering, voluntary activity, and a muscular rigidity of unknown origin.

The effects of temperature change on the blood as a physicochemical system have been described. The observed effects included those that could be predicted from our knowledge of the blood and others that reflect physiological responses to cold.

There is a trend toward acidosis, evidenced by a reduction commonly of one-quarter, in one case of one-half, in alkaline reserve. This reduction, measured at body temperature, occurs despite the fact that *in vitro* alkaline reserve increases with decreasing temperatures.

Hyperventilation provides additional evidence of decreasing alkaline reserve. Expired air may contain less than 1.5 per cent CO_2 when the arterial pCO_2 is only slightly reduced. Despite the hyperventilation and the presumed increase in alveolar pO_2, the arterial pO_2 decreases. These facts point to poorer diffusion in the lungs, a deduction supported by Walther's observation of pulmonary edema in hypothermic rabbits. Within the range of our observations, the shift in the oxygen dissociation curves to the left was enough to maintain a normal arterial saturation with oxygen.

Respiratory regulation remains effective even at 25°C. Not only the acid-base balance but perhaps also the arterial pO_2 is concerned in this function.

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IS HISTAMINE ABLE TO MAINTAIN AN AUGMENTED PEPSIN RESPONSE COMPARABLE TO THAT OF PILOCARPINE?

G. R. BUCHER, A. C. IVY AND J. S. GRAY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Up to the present time, the data relative to the effect of histamine on pepsin secretion have been obtained by the use of the single or double histamine test. Evidence has been presented (1) showing that the single or double histamine test yields data which have no value in critically determining whether the histamine produces an increased pepsin output by stimulation (2) or by a "washing-out" process (3, 4). It appeared to us that the "continuous histamine" test (5) would provide an ideal method of approach because a brisk continuous uniform secretion would insure against accumulation and "washing-out" processes, as well as against deleterious changes in the activity of pepsin due to wide variations of the pH of the gastric juice.

Accordingly, in this study we have used the continuous histamine test to determine whether relatively large doses of histamine given every 10 minutes will evoke and maintain in the vagotomized total stomach pouch of dogs a larger pepsin output than small doses of histamine. The study has been extended to ascertain how the pepsin output under "continuous histamine" compares with that of pilocarpine, a drug which every one agrees stimulates pepsin secretion (6).

EXPERIMENTAL PROCEDURE. Five dogs with vagotomized total-stomach pouches and thoroughly accustomed to experimentation were the subjects. All experiments were started after an over-night fast. The dose of histamine was adjusted to each animal so that in any series of tests, the volume-rate responses of the various animals were similar. No attempt was made in the pilocarpine series, however, to achieve such uniformity. The dose of pilocarpine was adjusted to the animal on the basis of 0.20 mgm. per 10 kilo body weight. The animals received a given dose of either or both drugs every 10 minutes throughout the experiment (see table 1). It was sometimes necessary to shift the histamine dose during an experiment to keep the volume-output within the desirable range. There was no consistency about the direction of the shifts or the time at which they occurred. The amount of the change in dose rarely exceeded

0.010 mgm.   Only two changes were made necessary in the pilocarpine dosage (see footnote, table 1).

All gastric drainage for the first 40 to 60 minutes was discarded.   By this time the secretory response to histamine had become steady, and that

TABLE 1

The dosage, in milligrams, of histamine and pilocarpine given subcutaneously every 10 minutes

ANIMAL	HISTAMINE		PILOCARPINE SERIES (c)
	Low series (a)	High series (b)	
	mgm.	*mgm.*	*mgm.*
C	0.020	0.080	0.20
M	0.025	0.100	0.34
F	0.025	0.090	0.10*
H	0.030	0.100	0.22
B	0.065	0.120	0.20*

* The dose for dog F had to be reduced from 0.21 mgm. pilocarpine to 0.10 mgm. during the first hour because of hemorrhage into the pouch.   Secretion failed during the fifth hour in dog B, and increasing the dose to 0.34 mgm. pilocarpine temporarily revived it.

TABLE 2

Key to the experiments performed

SERIES NAME	DURATION	NUMBER AND LENGTH OF PERIODS	DRUG AND DOSE (FROM TABLE 1)	NUMBER OF EXPERIMENTS
Low histamine	8 hrs.	(1)—8 hrs.	(a)	11
High histamine	8 hrs.	(1)—8 hrs.	(b)	10
Low-high-low histamine	7 hrs. and 20 min.	3		11
		P* (1) = 2 hrs.	(a)	
		P (2) = 2 hrs., 40 min.	(b)	
		P (3) = 2 hrs., 40 min.	(a)	
Pilocarpine	6 hrs.	(1)—6 hrs.	(c)	5
Histamine-pilo-carpine	7 hrs. and 20 min.	3		5
		P (1) = 2 hrs.	(a)	
		P (2) = 2 hrs., 40 min.	(a) plus (c)	
		P (3) = 2 hrs., 40 min.	(a)	

* The "P" indicates "period."   The extra 40 minutes were allowed for transition from one secretory rate to the other.

to pilocarpine had become sufficient to yield adequate samples.   The collection interval was 20 minutes.   All samples were analyzed for free and total acidity, and for pepsin by the hemoglobin method (1).   A Coleman glass electrode was used to determine pH in the low-high-low histamine series.   A key summarizing the important features of the 5

series of experiments is presented in table 2. In each series the same five animals were used.

The animals were encouraged to drink water during the experiments. In half of the experiments in the high histamine series, representing one on each animal, additional fluid and chloride was supplied by 400 to 600 cc. physiological saline given subcutaneously during the second to fifth
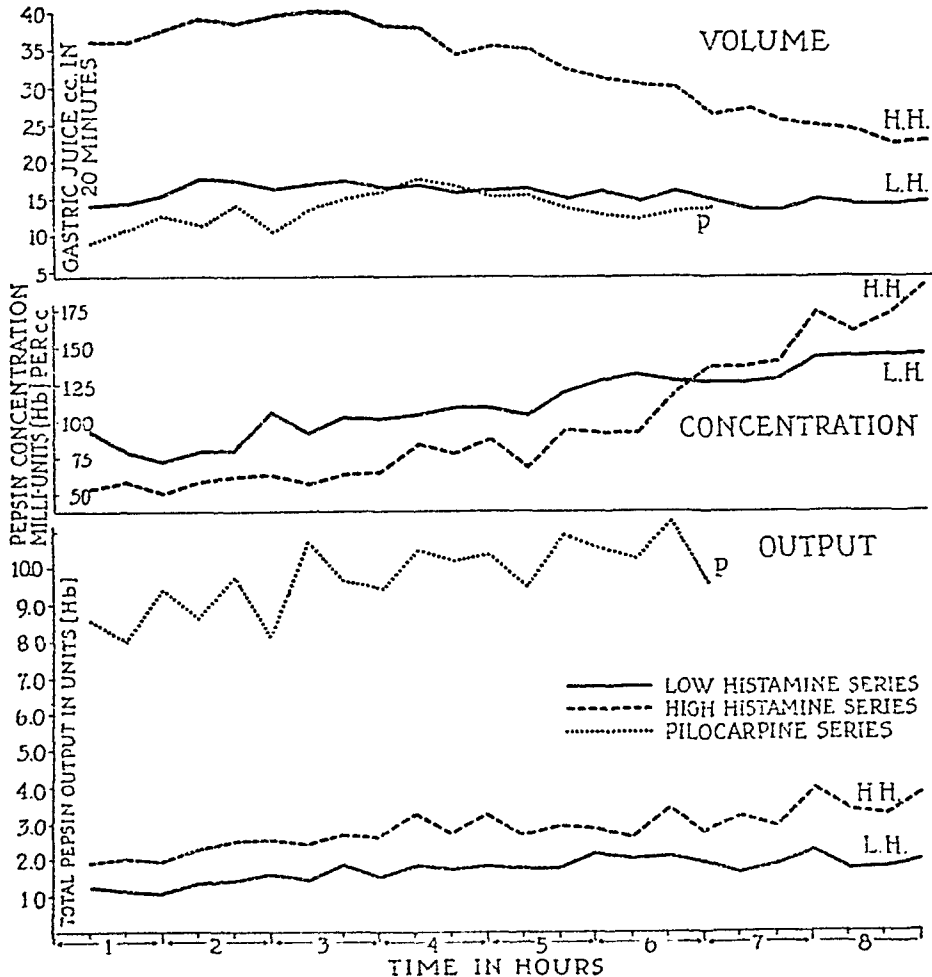
Fig. 1. Curves representing the arithmetical average of volume responses, pepsin concentration, and pepsin output in the low continuous histamine series, the high continuous histamine series, and in the continuous pilocarpine series.

hours. It was hoped that this measure would prevent the gradual decline in secretory rate which generally set in about the 4th hour in this series. This procedure tended to delay the onset of the decline but did not prevent it.

The observational unit employed in the graphs and in the statistical analysis has been the 20-minute outputs of volume and pepsin. The statistical methods used are the same as those employed previously (1).

DATA AND DISCUSSION.   *The high and the low histamine series.*   Results of the high and low histamine series are presented as mean curves of arithmetical averages in figure 1.   It is evident that the larger doses of histamine in the high series, in contrast to the low series, produced twice as much gastric juice, possessing a distinctly *lower pepsin concentration but a higher total pepsin output*.   This generalization is confirmed by statistical analysis of the 237 pairs of samples collected in the two series, and the data of 4 of the 5 animals when examined individually bear this out (table 3).   In one animal, dog B, the reversed response was observed.   This dog was unusually refractory to histamine during the time the low series tests were performed on him; he required over twice as much histamine as the other animals (table 1).   The reversed response of this one animal is completely lost when the data are statistically analyzed as a whole.   We may now

TABLE 3

Summary of pepsin-output in the high and low histamine series

ANIMAL	CORRELATION	PAIRS OF SAMPLES	MEAN MINUTE-OUTPUT OF PEPSIN, MILLI-UNITS (Hb)		SIGMA OF DIFFERENCE	CRITICAL RATIO
			High series	Low series		
C	+0.77	48	154 ±14.0	84 ±7.5	9.6	7.3
M	+0.16	48	124 ± 4.5	81 ±3.2	5.1	8.1
F	+0.45	45	234 ±14.5	155 ±9.6	13.4	5.8
H	+0.33	48	142 ± 5.0	55 ±3.3	5.0	17;4
B*	+0.72	48	45 ± 3.5	67 ±4.6	3.1	7.0*
All dogs	+0.62	237	140 ± 5.1	87 ±3.5	4.7	11.1

* Dog B was found to be significantly reversed.

conclude without equivocation that the *pepsin liberating mechanism*, as a rule, responds to histamine by an increased output of pepsin.

A second observation of importance is that in both series the pepsin concentration and pepsin output showed an unmistakable tendency to increase over the 8-hour period.   This is particularly true of the high histamine series.   The *definitely significant climb during the last 3 hours* of the high series, occurring in spite of a steadily diminishing volume-output, is especially significant when viewed in relation to the work of Bowie and Vineberg (7).   These workers, using dogs, under chloralose and urethane anesthesia and hourly doses of histamine (2 mgm. per 10 kilo body weight for 8 hours) found that the pepsin output which had been low from the beginning fell to zero at the end of the fourth hour.   The use of chloralose, which has been reported to inhibit the gastric secretory response to histamine, may be responsible for these results (8).   Lim and Liu (9), using the same hourly dose of histamine in Pavlov pouch dogs found that pepsin continued to be produced when the experiment was

continued as long as 24 hours, regardless of whether food and water were withheld during this time or not. The data of these latter workers also show that the average hourly outputs of pepsin tend to increase to a maximum about the 8th hour, after which they fall off somewhat to remain fairly uniform to the end of the 24-hour observation period.

In view of the belief that the parietal cell is non-fatigable (10), it is interesting to note that, in spite of fluid and chloride replacements when fairly intensive histamine stimulation is maintained, as in the high histamine series, the parietal cells manifest a "partial fatigue," so named by Lim and Liu (9), whereas the chief cells apparently do not. This is not manifest when less intensive stimulation is similarly prolonged, as in the low histamine series.
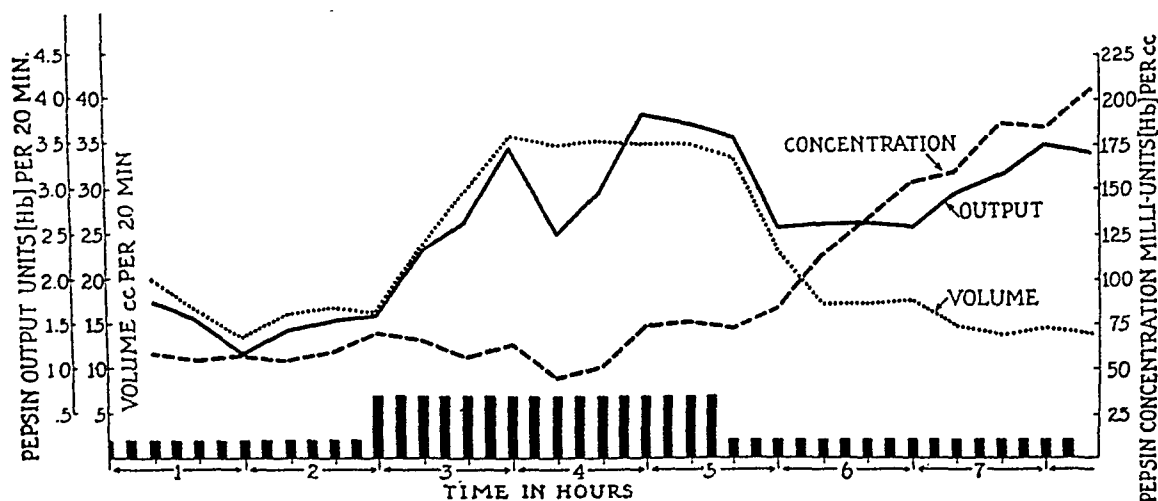
Fig. 2. Curves of the arithmetical averages of the responses in volume, pepsin concentration, and pepsin output in the low-high-low continuous histamine series.

Low-high-low histamine series. The ability of histamine in larger doses to evoke a significantly increased pepsin output is confirmed in the low-high-low histamine series of experiments (fig. 2). Excluding for the moment period 3 (fig. 2), the volume and pepsin outputs obtained in the first 2 periods are just what would be expected on the basis of the responses in the high and low histamine series shown in figure 1. The average outputs for the three periods are shown in table 4 and are compared with those of the low and high histamine series. The similarity of results is striking, except for the increased pepsin output during period 3.

The persistence of the augmented pepsin output in period 3, after the volume output had become adjusted to the lower histamine dosage, is an indication of an aspect of the pepsin secretory mechanism, as regards histamine, that has not been observed before, and which challenges explanation. It is perhaps an expression of the tendency of the pepsin out-

put to climb during continued uniform stimulation as observed in the high and low histamine series (fig. 1). We have at this time no explanation to offer for the sustained high peptic output after the histamine had been reduced and the volume response diminished by almost half, except to suggest that it is most likely due to an accumulative effect of histamine.

Comment. In the light of our observations, there is no evidence to support the view that histamine inhibits pepsin secretion (11). Neither do our data lend support to the view (3, 4) that the increase in pepsin output after histamine is due to a "washing-out" of previously secreted pepsin from the gastric tubules, for the continuous uniform secretion precluded accumulation of pepsin. Should it be suggested that temporarily increasing the histamine dosage increases the output of pepsin in the low-high-low histamine series, because more gastric glands were caused to secrete acid and hence more tubules were flushed out, we have merely

TABLE 4

Summary of mean minute-outputs of high histamine series, low histamine series and the low-high-low histamine series

NAME OF EXPERIMENT	VOLUME-OUTPUT	PEPSIN-OUTPUT
	cc.	*Milli-units (Hb)*
Low histamine series.....................	0.79	87.0
High histamine series....................	1.65	144.0
Low-high-low histamine series		
Period 1..............................	0.72	83.0
Period 2..............................	1.67	158.7
Period 3..............................	0.72	126.3

to observe how incompatible such a suggestion would be with the data in figure 2, especially period 3, wherein the output of pepsin was greater than at the start of the experiment when the same low histamine dose and volume response prevailed.

Whether the increased pepsin output after the injection of histamine is due to an actual direct stimulation of the chief cells by histamine cannot be absolutely decided by our data. The increased pepsin output rather than being due to a direct action of histamine on the chief cells might be due to the stimulation of the chief cells by the contact of acid with that portion of their surface exposed to the lumen of the glands. However, this is not likely since on resumption of low histamine dosage after the high histamine dosage there resulted a marked decrease in acid output without a proportional decrease in pepsin output. Unless one assumes that stimulation of the chief cells by acid contact continues after the flow of acid has markedly declined, it is difficult to avoid the conclusion that histamine directly stimulates the chief cells to secrete pepsin.

The pilocarpine series and the histamine-pilocarpine series.　The question now arises in regard to how the increased pepsin output caused by histamine compares with that obtained with a substance such as pilocarpine, which is admitted to act as a stimulant of the pepsin secreting cells.　A glance at figure 1 will convincingly show that when approximately equal volume responses are compared, the pilocarpine juice has about 5 times as much pepsin as the histamine juice.　Moreover, when pilocarpine was administered in addition to histamine (fig. 3), the pepsin response was only as great as if the pilocarpine had been given alone, while the volume
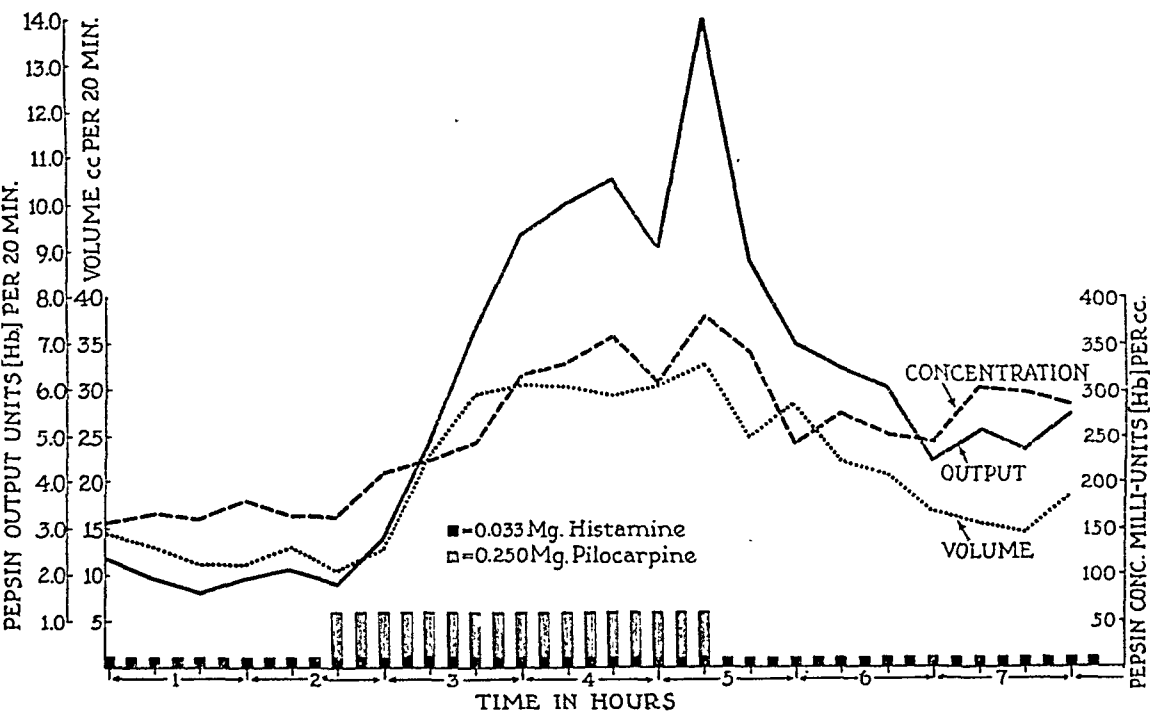
Fig. 3. Curves representing the arithmetical averages of volume, pepsin concentration, and pepsin output in the histamine-pilocarpine series.

responses showed an almost perfect summation of effect.　This is especially evident from the totaled outputs shown in table 5.

As in the case of "continuous histamine," the tendency for the pepsin output to climb during the course of the continuous pilocarpine experiment is evident (fig. 1).　The average minute-output for the last 4 hours of the pilocarpine series is 508.0 milli-units of pepsin, exactly the value achieved when the same amount of pilocarpine was administered with histamine, which alone was maintaining a pepsin output of 98.0 milli-units per minute.

When the pilocarpine was discontinued, there was an immediate and sudden fall in the pepsin output to a constant level which was almost three times as great as during the first period under the same degree of histamine stimulation.　The suggestion of Necheles *et al.* (14) and Ihre (3)

that histamine and pilocarpine are synergistic as long as given together and that a temporary exhaustion response follows the cessation of either of the drugs is not sustained by this evidence. It should be pointed out, however, that a dosage factor may be involved in the question of synergism. After cessation of the pilocarpine, the volume response returned to the precise level under histamine alone, and the pepsin output, though less than in period 2, was markedly increased over histamine alone (figs. 1 and 2). If the tendency for pepsin output to continue at a high level after discontinuance of pilocarpine is interpreted as due to a continuance of the stimulating action of the drug, then the same interpretation may be applied to the same tendency manifested by histamine.

Evidence from experiments on humans has been reported (12, 13) which indicates that histamine is neither inferior nor superior to pilocarpine

TABLE 5

Summary of the mean minute-outputs of volume and pepsin in the pilocarpine series and in the histamine-pilocarpine-histamine series

NAME OF SERIES	VOLUME-OUTPUT	PEPSIN-OUTPUT	TYPE OF STIMULATION
	cc.	*Milli-units (Hb)*	
Pilocarpine series...............	0.68	475.0	Pilocarpine (c)
Hist.-Hist.P.-Hist.			
Period 1.....................	0.82	98.0	Low histamine (a)
Period 2.....................	1.54	507.0	Low histamine (a) plus pilocarpine (c)
Period 3.....................	0.90	273.0	Low histamine (a)

in evoking gastric secretion as measured by volume, acidity and pepsin output. Our results on the dog do not permit us to agree with such a view. Although a species difference may play some part in this discrepancy, we believe that their use of the double histamine test and its inherent errors (1) is the most responsible factor.

SUMMARY. 1. Pepsin output is not inhibited or diminished during the course of an eight-hour "continuous histamine" test (a small dose of histamine subcutaneously every 10 min.) in dogs with pouches of the entire stomach and the vagal innervation cut. Indeed, it tends to increase with time especially when higher dosages of histamine are used.

2. The minute-output of pepsin in response to large doses of histamine such as 0.10 mgm. given every 10 minutes is at all times significantly larger than that in response to smaller doses such as 0.025 mgm. given every 10 minutes.

3. If during the course of a submaximal "continuous histamine" test (0.025 mgm. every 10 min.) the histamine dose is increased (0.10 mgm. histamine every 10 min.), the pepsin output will increase.

4. If during the course of a submaximal "continuous histamine" test the·dose is increased for a period and then decreased again to the submaximal level, the output of pepsin continues at a higher level than during the first submaximal dosage period; i.e., the effect of the higher dose of histamine in increasing pepsin output tends to continue after resumption of a lower dose of histamine.

5. Pilocarpine given subcutaneously every 10 minutes in doses which produce a volume-rate response comparable to a low dose of histamine yields a pepsin output five times that of the histamine. The output, as with histamine, tends to increase during the course of a 6-hour experiment.

6. When pilocarpine and histamine are given together a summation of the effect of the two drugs is observed in the volume output, but the pepsin output is typical of pilocarpine when administered alone. The usual effect of histamine, which was continued after cessation of the pilocarpine, was unchanged, but the pilocarpine effect on pepsin output continued as in the case of histamine (see conclusion 4).

CONCLUSION

Under the conditions of our experiments, if pilocarpine is considered to stimulate directly the pepsin secreting cells, histamine does likewise on the basis of similar evidence and analogous deductions. The only difference is that with similar volume outputs of juice, pilocarpine yields considerably more pepsin than does histamine.

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STUDIES ON THE DISTRIBUTION OF RADIOACTIVE FLUORIDE IN THE BONES AND TEETH OF EXPERIMENTAL ANIMALS[1]

J. F. VOLKER, R. F. SOGNNAES AND B. G. BIBBY

From the Division of Dental Research, the University of Rochester School of Medicine and Dentistry, Rochester, New York[1]

Investigations of various skeletal abnormalities including chronic fluorosis (De Eds, 1933; Peirce, 1939) dental caries (Cox, 1940; Hodge, 1940) and rickets (Morgareidge and Finn, 1940) all point to a significant relationship between fluoride and the inorganic metabolism of bones and teeth. One of the great difficulties in fluoride studies has been the exacting methods necessary for the chemical determination of fluoride in the calcified tissue. Recently the radioactive isotope F^{18} has been used for in vitro mineral metabolism studies (Volker, Hodge, Wilson and VanVoorhis, 1940). To date in vivo researches using the same agent have not been attempted. Since it seemed probable that such an experiment might increase our knowledge of the biochemistry of fluorides, a series of experiments with the radioactive isotope was attempted.

PROCEDURE. Radioactive fluorine was obtained from the Department of Physics of this University through the courtesy of Dr. S. N. VanVoorhis. The isotope was prepared by bombarding the oxygen in water molecules by the proton beam of the cyclotron. The nuclear reaction is as follows:

$$_8^{18}O + _1^1H \longrightarrow _9^{18}F + _0^1n$$
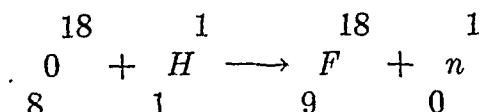
The unstable isotope has a half life of 112 minutes and reverts to O^{18} by the emission of a positron as follows:

$$_9^{18}F \longrightarrow _8^{18}O + _1^0e$$
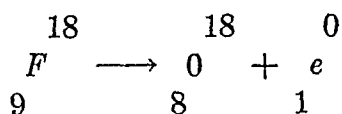
Two groups of experimental animals were used in this study. The first group was composed of 5 adult rats, weighing approximately 250 grams

each, and the second group 4 adult cats weighing from 2.6 to 4.0 kgm. Rats 4 and 5 were suffering from chronic fluorosis induced by the long time ingestion of a diet containing approximately 300 p.p.m. of fluoride. Animals in the first group were given intraperitoneal injection of 2 to 4 mgm. of fluorine as sodium fluoride in physiological saline plus a known amount of marked fluoride. Thirty-five minutes after the administration of the fluoride, the rats were sacrificed by decapitation and a sample of blood collected and oxalated. Samples of the molar crown, femur shaft, inferior and superior maxillae, incisor tips, and whole incisor minus the incisor tip were cleansed of the adhering tissue, broken up, weighed and dissolved in 6 N/HCl. An attempt was made to remove the pulp tissue from the molar crowns without disturbing the coronal dentin. Two cubic centimeter aliquots of the dissolved tissues were placed in a counting cup of the Geiger-Müller scale-of-4 counter and the number of counts determined for duplicate or triplicate five-minute periods. At suitable intervals the radioactivity of the standard radioactive fluoride solution was determined, as well as the background count and the counter sensitivity.

Animals in the second group were given intravenous injections of 10 mgm. of labeled fluoride in physiological saline solution. The submaxillary saliva was collected from the cannulated duct, the salivary flow being increased by electrical stimulation of the chorda tympani nerve. Periodic blood samples were also taken. Both these phases of the study were carried out by J. H. Wills of the Department of Physiology and are reported by him in detail elsewhere (Wills, 1940). After the collection of the last blood sample, the animal was immediately sacrificed and samples of whole teeth, depulped cuspid crowns, superior and inferior maxillae, femur shaft, intestinal washings, and salivary glands were collected. In order to make certain the complete removal of the residual pulp tissue from the cuspid crowns, the walls of the pulp chambers were cleaned with a dental burr. The calcified tissues were prepared for counting as previously described. The soft tissues were ground in a mortar with sand and extracted with saline solution. In each of the four cats the intact bladder was removed and aliquots of urine taken. In no case did urination occur during the experimental period. Radio fluoride determinations were made by the same procedure as used for the group 1 samples.

FINDINGS. The results obtained with the group 1 rats may be seen in table 1. It will be noted that the concentration of the fluoride in the blood at the time of death shows marked variation. Since the blood level of the fluoride is probably directly dependent on the rate of absorption from the gut, it would appear that this property is quite variable in rats when the fluoride is administered by intraperitoneal injection. These variations are most likely to be found in short-term experiments such as those necessitated by problems involving short half-life isotopes. This same factor,

the blood level of the fluoride, undoubtedly influences the rate and percentage of absorption by the various calcified tissues. The percentage of the total dose per gram of tissue figures indicate that the calcified tissues take up fluoride from the circulating blood; the amount of fluoride in the skeletal tissues is roughly parallel to the adequacy of the blood supply with no detectable amounts of fluoride being present in the incisor tip. The figures for the incisor minus the incisor tip, are slightly below those observed for whole bone, but since these samples contain at least half the total amount as fully calcified and erupted tooth substances, the percentage of total dose per gram of tissue of the remaining half (actively calcifying root) is probably in excess of that observed for jaw bone or femur. This would be expected since, in addition to the possibility of a

TABLE 1

Radioactive F distribution in the rat

RAT NUMBER	DOSE OF FLUORIDE	PER CENT OF TOTAL DOSE PER GRAM OF TISSUE				
		Blood	Molar crown	Femur shaft	Combined maxillae	Incisor minus incisal third
	mgm.					
1	4	0.128	0.294	0.371		
2	4	0.370	0.435	0.647		
3	2	0.208	0.234	1.327	1.227	1.197
4*	2	0.128	0.229	0.901	0.993	0.786
5*	2	0.170	0.421	0.716	0.910	0.867

* Fluorosed.

Incisor tip *gave* no count except in rat 2 where a portion of the middle third was included.

Salivary gland rat 2 (800 mgm.) gave 0.098 per cent of total dose per gram of tissue.

normal exchange reaction, the process of calcification is proceeding at an accelerated rate in this area. The presence of detectable amounts of fluoride in the cleansed molar crowns was a consistent finding and probably indicates that fluoride is being deposited in that part of the dentin approximating the pulpal tissue.

No striking difference in F metabolism was evident in a comparison of the normal and fluorosed animals. The results obtained with group 2 cats may be seen in table 2. Cats 1 and 2, which were allowed to live for approximately two hours following the administration of fluoride, may be compared with cats 3 and 4 which were sacrificed after a wait of approximately 30 minutes. It will be noted that the blood fluoride level in cats 1 and 2 was much less than that of the calcified tissue, whereas in cats 3 and 4 the reverse was true. This would seem to indicate that the injected fluoride rapidly leaves the circulating blood and becomes deposited in the

skeletal tissues.   As previously noted in the group 1 experiments, the percent of total dose radio fluoride per gram tissue varies with the extent of contact of decalcified tissue with the circulating blood, being greatest in the bone and negligible in the depulped cuspid crowns.   The differences observed in the two types of bone, namely, the femur and mixed jaw bone samples, may be due in part to their anatomical dissimilarity, the maxillae being flat or skull bones and the femur a long bone.   Also it is conceivable that the rate of fluoride deposition may follow a different curve for various bones, depending on the adequacy of the intraosseous circulation.   The failure to demonstrate a similar difference between the rat maxillae and femur shaft needs further investigation.   Two possible factors may be cited in this connection.   First, a comparatively greater portion of the

TABLE 2

Radioactive F distribution in the cat

CAT NUMBER	WEIGHT	PER CENT OF TOTAL DOSE OF FLUORIDE PER GRAM OF TISSUE				PER CENT TOTAL DOSE F IN SALIVA	PER CENT TOTAL DOSE F IN URINE
		Blood	Whole tooth	Combined maxillae	Long bone		
	kgm.						
1	2.8	0.015	0.030	0.095		0.1102	14.4
2	2.6	0.016	0.032	0.101	0.037	0.0415	14.6
3	4.0	0.060	0.014	0.030		0.0877	22.7
4	3.5	0.064	0.015	0.038	0.020	0.0540	10.4

Each cat received 10 mgm. of NaF plus the radioactive isotope.

Samples of intestine, salivary gland and cuspid tooth crowns contained insufficient fluoride for estimation.

Time of salivary fluoride secretion in cats 1, 2, 3, 4 was 117, 106, 19 and 21 minutes respectively.

rat femur was taken for analysis than was used in the cat experiments. This was necessitated by the relatively small size of the rat's femur. Second, a greater dose of fluoride per gram of body weight was given to the rats than was administered to the cats.

The percentage of the total doses of fluoride secreted in the saliva was calculated from the data of Wills.   These figures and those obtained for the percentage of the total dose excreted in the urine, are approximately the same for the one-half and the two hour animals, indicating that the greater proportion of the salivary and urinary fluoride was excreted within the first 30 minutes of the experimental period, when the blood level of fluoride is correspondingly high.

DISCUSSION.   It will be noted that the actively calcifying portions of the rat incisors show the greatest deposition of fluoride.   This is in agreement with the observation that the incisors of the rat are extremely susceptible

to disturbances in calcification and pigmentation, as seen in chronic fluorosis, and is also in keeping with the chemical finding that the fluoride content of the rat incisor can be markedly increased on a high fluoride diet (Hodge, Luce-Clausen and Brown, 1939).

The high fluoride deposition in the bony skeleton is probably significantly related to the observation that the addition of fluoride to a rachitogenic diet decreases the severity of experimental rickets (Morgareidge and Finn, 1940).

The small fluoride secretion in the saliva and the lack of appreciable fluoride deposition in the incisal third of the rat incisor and depulped cuspid crowns may help to clarify the mechanism by which fluoride reduces caries incidence. In this connection we have found that the average fluoride secretion in the saliva of the group 2 animals totaled only one one-thousandth of the injected dose. Since the two-hour excretion approximated that of the half-hour excretion, it would appear that comparatively little fluoride was secreted in the saliva after the first half-hour. It should be remembered that the injected dose of fluoride (10 mgm.) is probably in excess of the amount found in the daily diet of humans, and that the experimental animals used were approximately $\frac{1}{20}$ the size of an average adult human male. The failure to find significant amounts of the labeled fluoride in the dental tissues well removed from the tooth pulp, does not support the possibility that fluorine may be deposited via the circulation in the dental enamel. This point is of particular interest to workers in caries research, since it has been shown that the fluoride content of non-carious enamel is significantly higher than that of carious enamel, although no such difference exists between carious and non-carious dentin (Armstrong and Brekhus, 1938). However, the possibility still exists that the ingested fluoride is first stored in the bones and may later be released to the circulation where it could conceivably be deposited in the more inert portion of the teeth. Unfortunately, the short half-life of the radioactive isotope will not permit studies to test that possibility.

SUMMARY

Radioactive fluorine was given by intraperitoneal injection to five rats and by intravenous injection to 4 cats. Concentration of the blood fluoride fell rapidly with a corresponding rise in the calcified tissue fluoride. The radioactive fluoride concentration of the various skeletal tissues was approximately directly proportional to their proximity to the circulating blood. Urinary excretion and salivary secretion of the isotope occurred in appreciable amounts only when the blood concentration was elevated.

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THE NATURE AND LOCATION OF THE "SPHINCTER MECHANISM" IN THE LIVER AS DETERMINED BY DRUG ACTIONS AND VASCULAR INJECTIONS

L. J. DEYSACH

From the Department of Physiology, Marquette University School of Medicine, Milwaukee, Wisconsin

Ever since the early work of Stolnikow in 1882, the rôle of the liver as a reservoir for blood has been the subject of numerous investigations. Although it is generally conceded that the liver exerts a control over the blood flow through it, knowledge regarding the mechanism or mechanisms which contribute to the partial or total retention of blood in this reservoir is still fragmentary and conflicting.

The present investigation was undertaken in order to secure further information regarding the nature and location of the mechanism which controls the outflow of blood through the hepatic veins. Two methods of study were employed in this investigation: 1, perfusion of excised livers with various drug solutions (adrenalin, pilocarpin, physostigmin, atropine and acetyl-beta-methylcholine chloride); and 2, detailed microscopic examination of injected and cleared specimens obtained from livers previously perfused with the various drugs.

An important part of the discussion concerns two angioarchitectural features which seem to have entirely escaped the attention of earlier investigators. One of these features is the artery-like nature of the sublobular veins, and the other one is the structure which will be referred to as the "small sluice channel". The "small sluice channels" are peculiar side branches of the sublobular veins (figs. 7, 8, 9, 10, 11 and 12). They, together with the relatively thick walls of the sublobular veins, form a complex or multiple "sluice valve" which is probably the most important part of the mechanism which regulates the outflow of blood from the liver.

MATERIAL AND TECHNIQUE. A comparative study was made of the livers of many laboratory and zoo mammals, but the results herein reported were obtained principally from surviving livers of adult cats and rabbits. The common laboratory animals were all killed by a sudden blow on the occiput. The others were killed by bleeding. In each case the liver was immediately exposed through a midline abdominal incision, the arteries to the intestines and stomach were ligated, and the lower end of the esoph-

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agus was doubly tied and cut.   The portal branches were ligated and a glass cannula was inserted into the portal vein as closely as possible to the hilus of the liver.   After removing the stomach and intestines, the hepatic artery was ligatured and the abdominal vena cava was tied and then severed close to its entry into the liver.   The thorax was opened and a second glass cannula was tied into the thoracic vena cava close to the diaphragm.   The entire liver, with the diaphragm attached, was then removed and placed into an air plethysmograph which in all essential respects was like that described by Bauer, Dale, Poulsson and Richards (1932).   The cannula in the portal vein was connected by means of rubber and glass tubing to a constant level reservoir containing warmed (39°C.) oxygenated dextrose-free Locke's solution.   This reservoir was fixed at an appropriate level above the liver.   All drug solutions were slowly administered from a burette or a series of burettes directly into the perfusion stream at a point close to the cannula which had been inserted into the portal vein.   Solutions of varying H-ion concentrations were used in these experiments.   It was observed that the most constant results were obtained when the pH of the perfusion fluid ranged between 7.8 and 8.15. An automatic tilting bucket was used to record the outflow of liquid from the liver.

At the completion of each perfusion experiment the liver was removed from the plethysmograph and the blood vessels in the organ were then injected with India ink alone, with India ink and vermilion cinnabar, or with vermilion cinnabar alone.   Whenever a differential injection of the blood vessels was made, India ink was injected in a rhythmic manner and under low pressure (5 to 15 mm. of mercury) into the cannula which had been inserted into the inferior vena cava and then vermilion cinnabar (in water) was injected into the cannula inserted into the portal vein.   All injected livers were cleared and otherwise prepared according to the methods described by Swindle (1935).   The tissue was later sectioned and then examined with a binocular microscope.   Some of the sections were mounted in dammar and photographed (figs. 7, 8, 9 and 10).   Other sections were stained in order to determine some of the histological features of the blood vessels (figs. 11 and 12).   All of the photomicrographs (figs. 7, 8, 9, 10, 11 and 12) were obtained by using the Eastman D-C Ortho-photographic plate with transmitted light.

RESULTS.   *Effect of adrenalin.*   Adrenalin chloride solution (P.D. & Co.) was used in quantities of 25 cc. of a 1 in 400,000 and a 1 in 60,000 solution. When the weaker solution of adrenalin was perfused by way of the portal vein through the liver of a rabbit, the outflow from the organ increased from 22.819 cc. per 100 grams per minute to 28.91 cc. per 100 grams per minute (fig. 1).   This was an increase in outflow of 26.69 per cent.   The total liver volume diminished 3.62 cc.   Perfusion of the rabbit liver with

the 1 in 60,000 solution reduced the outflow from 26.77 cc. per 100 grams per minute to 10.12 cc. per 100 grams per minute, a decrease of 62.19 per cent (fig. 2). The total liver volume diminished 3.75 cc. Such contrasted
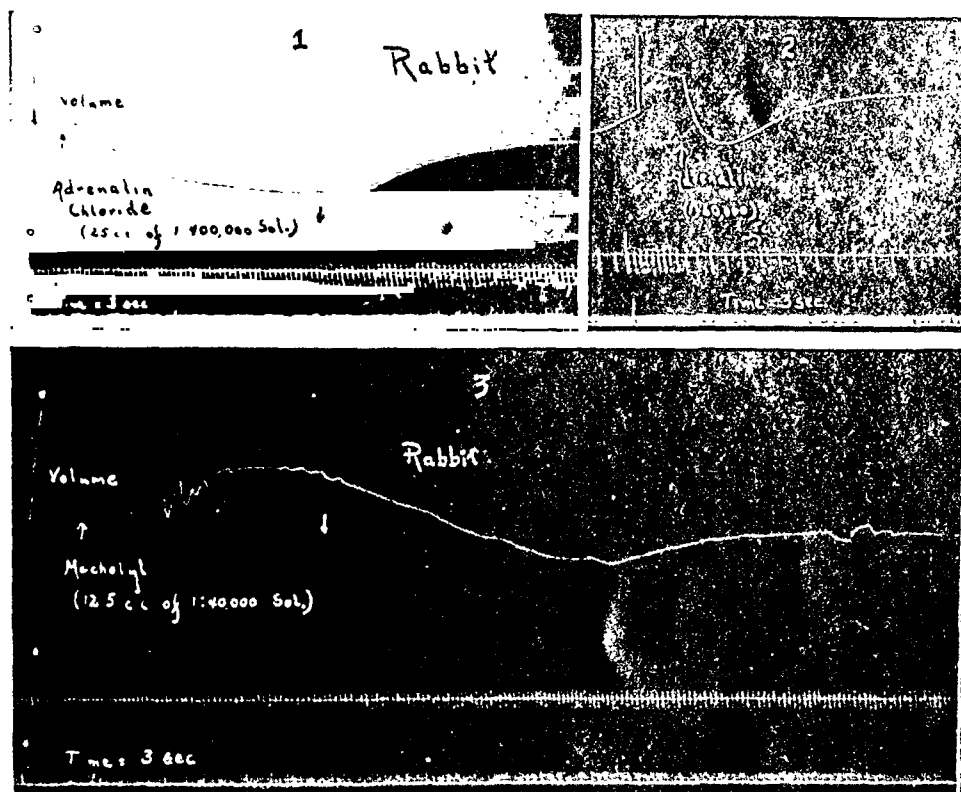
Fig. 1. Upper tracing, volume curve of the excised, surviving liver of an adult rabbit. Lower tracing, outflow of liquid through the inferior vena cava, each space between the perpendicular marks representing the outflow of 5 cc. of liquid. Perfusion of the liver by way of the portal vein with 25 cc. of a 1:400,000 adrenalin chloride solution reduced the liver volume 3.5 cc., and increased the outflow of liquid from the inferior vena cava 26.69 per cent.

Fig. 2. Same as figure 1 except that a 1:60,000 solution of adrenalin chloride was perfused through the liver. The liver volume was reduced 3.75 cc., and the outflow of liquid from the inferior vena cava was diminished 62.19 per cent.

Fig. 3. Upper tracing, volume curve of the excised, surviving liver of an adult rabbit. Lower tracing, outflow of liquid through the inferior vena cava, each space between the perpendicular marks representing the outflow of 4.5 cc. of liquid. The addition of 12.5 cc. of a 1:40,000 acetyl-beta-methylcholine chloride solution to the perfusion fluid increased the liver volume and diminished the outflow of liquid through the inferior vena cava.

effects of increased outflow after the smaller dose of adrenalin and decreased outflow after the larger dose of adrenalin were observed in experiments on nine cats, thirty-five rabbits and two vervet monkeys. It is of interest to note that the larger dose of adrenalin did not have a greater weakening effect on the resistance offered to the outflow than the smaller

dose, as shown by the almost identical diminution in liver volume in both instances.

The injection of India ink by way of the inferior vena cava into livers which had been perfused with a small dose of adrenalin always produced very complete injections of the blood vessels in the organ, and the injection medium flowed freely out of the portal vein. In no instance was it necessary to use an injection pressure exceeding fifteen millimeters of mercury. When the blood vessels in any of the livers which had been perfused with a large dose of adrenalin were injected by way of the inferior vena cava, the liver expanded enormously and the outflow from the portal vein was observed to be very meager. Injection of the blood vessels of a liver by way of the portal vein, the organ having been previously perfused with a large dose of adrenalin, always required an injection pressure exceeding fifteen millimeters of mercury in order to force the injection medium into the liver lobules. This was not the case, however, when India ink was injected by way of the portal vein into livers which had been previously perfused with a small dose of adrenalin. In all instances India ink always flowed readily into the liver lobules even when the injection pressure was very low (5 mm. of mercury).

Effect of acetyl-beta-methylcholine chloride. Swelling of the liver and restriction of the outflow was regularly observed in rabbits (fig. 3), cats (fig. 6) and vervet monkeys when acetyl-beta-methylcholine chloride ("Mecholyl"-Merck) was perfused through the organ by way of the portal vein. As shown in figure 6, when 25 gamma of acetyl-beta-methylcholine chloride were perfused through a cat's liver, the liver volume increased 8.75 cc. and the hepatic outflow was reduced from 12.12 cc. per minute to 2.64 cc. per minute, a reduction of 78.2 per cent. The administration of 2 cc. of a 0.1 per cent solution of atropine sulphate while the liver was enormously expanded promptly checked and reduced the expansion of the organ, and the hepatic outflow became markedly accelerated.

The injection of India ink by way of the inferior vena cava into a liver which had been previously perfused with "Mecholyl" always produced very incomplete injections of the blood vessels in the organ (fig. 7). In many instances an injection pressure exceeding 150 mm. of mercury was found necessary in order to produce a free flow of the ink through the portal vein. Attempts to inject the liver by way of the portal vein always resulted in marked expansion of the organ and a meager flow of the injection medium from the inferior vena cava. When atropine sulphate had been perfused through the liver prior to the injection of the blood vessels in the organ, little or no resistance was offered to the flow of the ink. Only low injection pressures (5-10 mm. of mercury) were found necessary for thorough injection of these atropinized specimens.

Effect of physostigmin and pilocarpin. The results obtained by perfusing

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cat and rabbit livers with either pilocarpin or physostigmin were in all essential respects similar to those obtained when acetyl-beta-methyl-
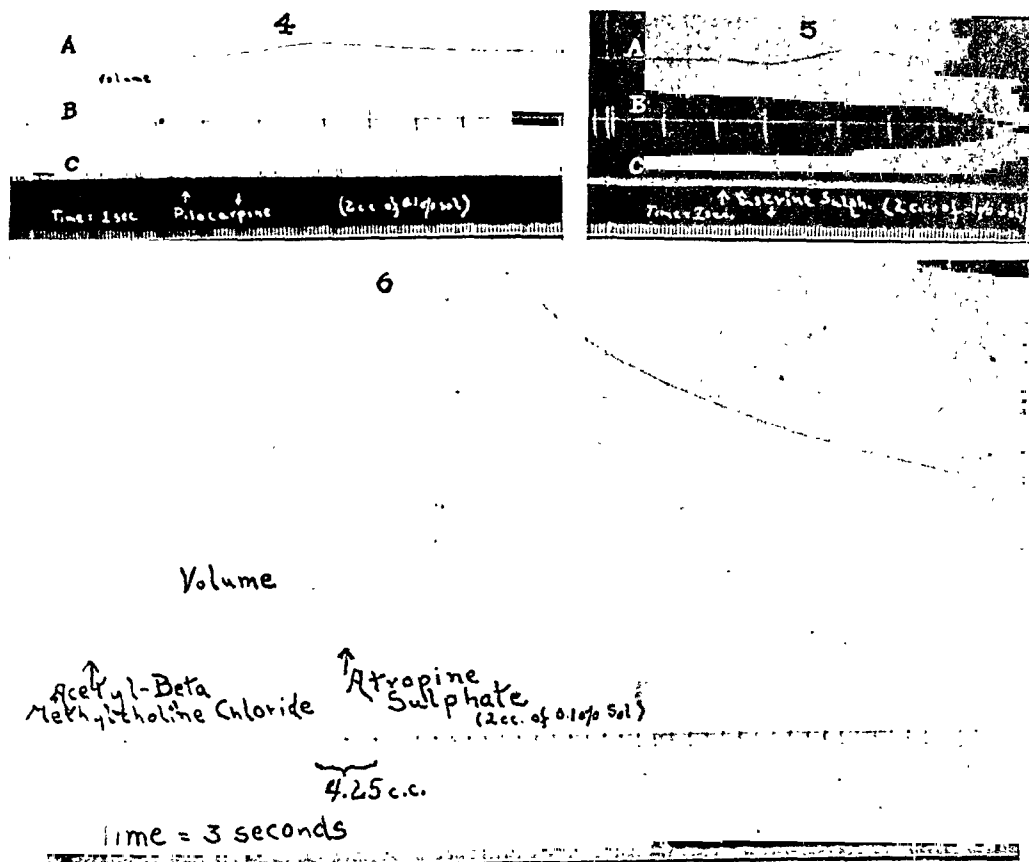
Fig. 4. Plethysmographic tracing obtained from the excised, surviving liver of an adult rabbit. The addition of 2 cc. of a 0.1 per cent pilocarpin hydrochloride solution caused an increase in liver volume and a decrease in liver outflow. A, volume curve of the liver; B, outflow of liquid from the inferior vena cava measured in 3.5 cc. volumes; C, the outflow in drops.

Fig. 5. Plethysmographic tracing from the excised, surviving liver of an adult rabbit showing the effect of perfusing 2 cc. of a 0.1 per cent eserine sulphate solution through the liver by way of the portal vein. The liver volume increased and the outflow from the inferior vena cava was reduced 29.98 per cent.

Fig. 6. Upper record, volume curve of the excised, surviving liver of an adult cat. Lower record, outflow of liquid through the inferior vena cava measured in 4.25 cc. volumes. When 25 gamma of acetyl-beta-methylcholine chloride solution were perfused through the liver by way of the portal vein, the volume of the liver increased 8.75 cc. and the outflow diminished 78.2 per cent. The addition of 2 cc. of a 0.1 per cent atropine sulphate solution checked and reduced the expansion of the organ, and the hepatic outflow became markedly accelerated.

choline chloride was added to the perfusing fluid. In all instances there was swelling of the liver and restriction of the outflow of liquid through the inferior vena cava. As shown in figure 5, when 2 cc. of a 0.1 per cent

solution of physostigmin sulphate were administered by way of the portal vein, the flow from the rabbit liver was reduced from 14.74 cc. per 100 grams per minute to 10.32 cc. per 100 grams per minute, a decrease of 29.98 per cent. Similarly pilocarpin hydrochloride (fig. 4) reduced the outflow from the rabbit liver from 16.51 cc. per 100 grams per minute to 12.51 cc. per 100 grams per minute, a decrease of 24.22 per cent.

The results of injection of the blood vessels in livers perfused with either pilocarpin or eserine were similar to those obtained after the liver had been perfused with "Mecholyl." In no instance, however, was it necessary to exert a pressure greater than 40 mm. of mercury to obtain a free flow of liquid through the organ.

Microscopic examination of injected and cleared specimens. Detailed microscopic examination of the blood vessels in livers which have been perfused with either atropine or small doses of adrenalin reveals that the sublobular veins are markedly dilated and that they possess an unusually large number of peculiar side branches which are smaller but otherwise very similar morphologically to the central veins in the liver lobules. Each of these unusual side branches arises from the confluence of many ordinary sinusoidal capillaries. They penetrate more or less perpendicularly through all of the tunics of the sublobular vein (figs. 9 and 10). In this respect, these small side branches of the sublobular vein resemble the "small sluice channels" which were recently described in the erectile tissue of the penis (Deysach, 1939).

Relatively few or occasionally none of the unusual side branches can be observed piercing the sublobular veins in livers which have been perfused with acetyl-beta-methylcholine chloride, eserine or pilocarpin (figs. 7 and 8). The distribution of India ink in the liver lobules of these livers is such that only the sinusoidal capillaries in the immediate vicinity of the central veins are thoroughly filled. In contrast to this observation, however, whenever atropine or a small dose of adrenalin is perfused through the liver prior to the injection of the blood vessels, all of the sinusoidal capillaries in the liver lobules invariably become filled with the India ink (figs. 9 and 10).

Microscopic examination of injected and stained specimens obtained from the livers of cats, vervet monkeys and rabbits reveals that the hepatic veins and their branches are relatively thick walled and muscular (figs. 11 and 12), whereas the portal vein and its branches are very thin walled and poorly supplied with muscle tissue. The walls of the hepatic venous ramifications (e.g., the sublobular veins) are made up primarily of smooth muscle fibers, collagenous fibers, fibroblasts and a small amount of elastic fibers. The walls of the portal venous ramifications are made up of smooth muscle fibers, which are predominantly circularly arranged, elastic fibers, and a few collagenous fibers and fibroblasts. The smooth muscle fibers in the sublobular veins are arranged both longitudinally and circularly.
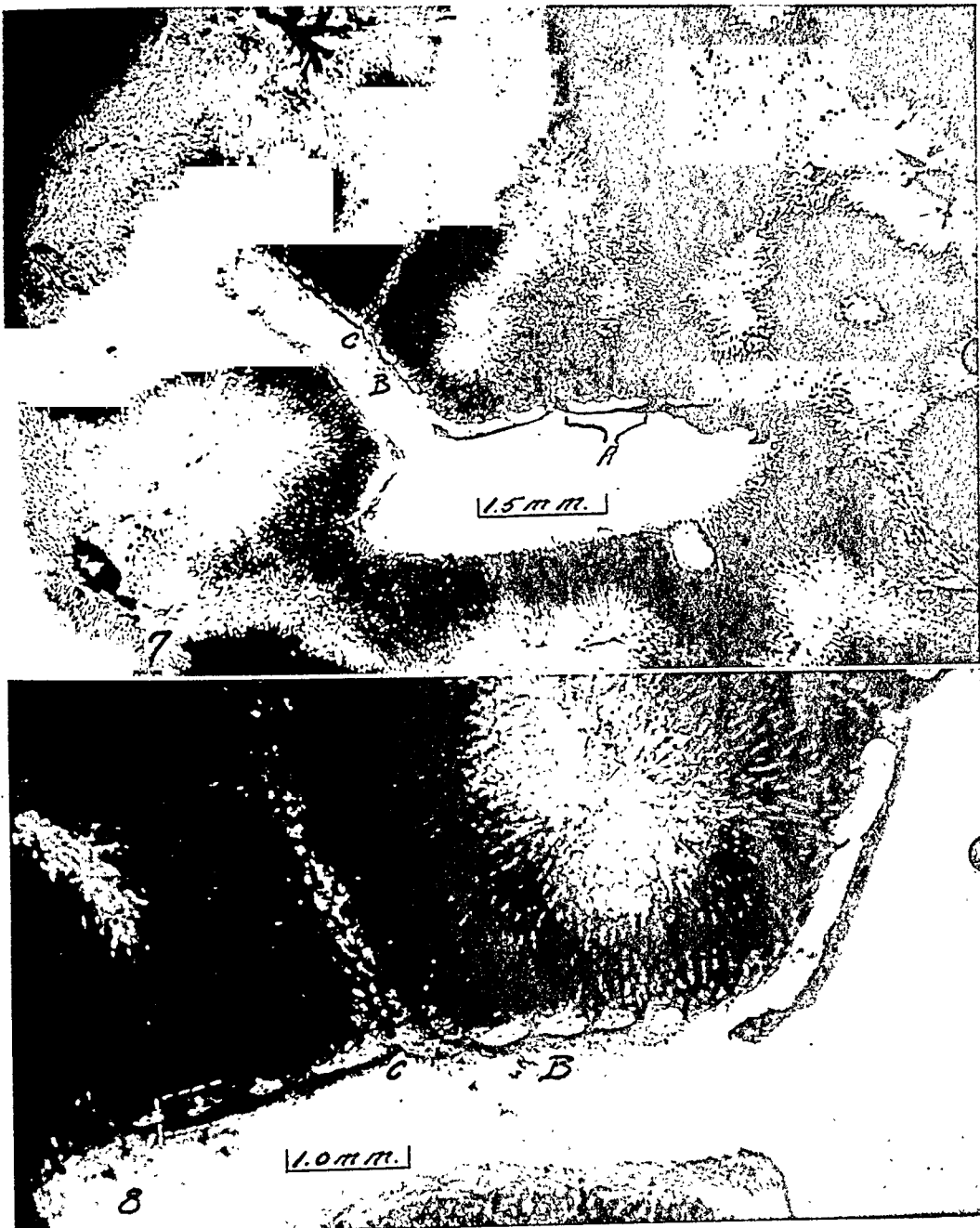
Fig. 7. Photomicrograph of a section obtained from an injected and cleared liver of an adult rabbit after the organ was perfused with 12.5 cc. of a 1:40,000 solution of acetyl-beta-methylcholine chloride. India ink was injected in a rhythmic manner and under low pressure into the inferior vena cava, and vermilion cinnabar (in water) was injected into the portal vein. A, small sluice channels closed; B, small sluice channels open; C, large, open sluide channels (central veins).

Fig. 8. Same as figure 7 except a relatively greater magnification was used in an effort to show the nature of the small sluice channels. The thickness of the wall of the sublobular vein is outlined by the broken lines made on the photographic print with pen and ink. It is interesting to note that the sinusoidal capillaries beyond the widely open sluice channels are thoroughly filled with ink.

Fig. 9. Photomicrograph of a section obtained from an injected and cleared liver of an adult cat after the organ was perfused with a 0.1 per cent atropine sulphate solution. The technique used in preparing this specimen was the same as for figure 7. Due to the fact that numerous small sluice channels were widely open, the sinusoidal capillaries in the liver lobules became thoroughly filled with the India ink solution.

Fig. 10. Same as figure 9 except a relatively great magnification was used in an effort to show the nature of the small sluice channels. All of the sublobular veins were markedly dilated throughout the specimen from which this section was obtained. Due to the relaxation of the musculature in the walls of the sublobular veins, India ink flowed freely through the widely open sluice channels and completely filled the sinusoidal capillaries in all of the liver lobules.
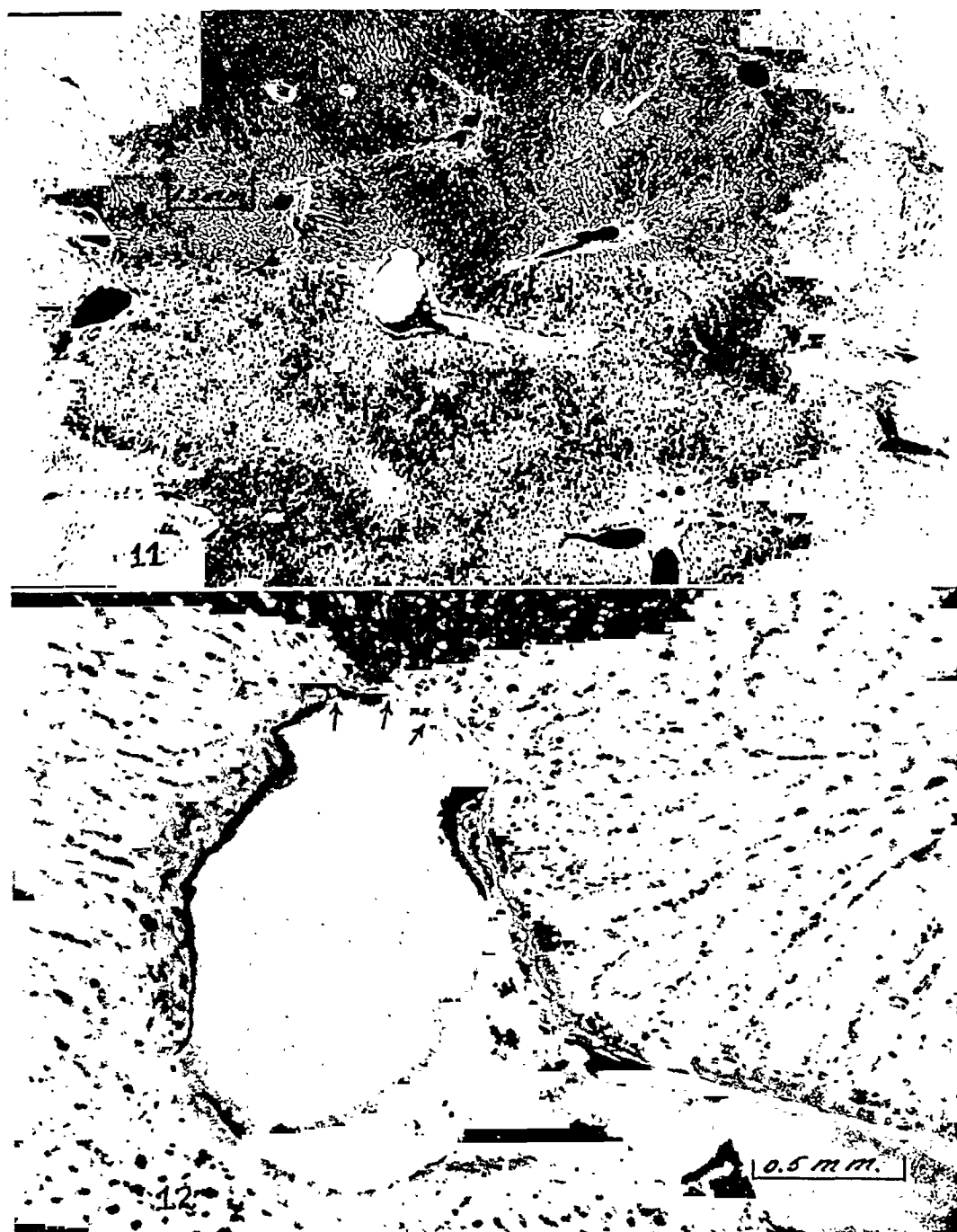
Fig. 11. Photomicrograph of an injected and stained specimen obtained from the liver of an adult rabbit after 12.5 cc. of a 1:40,000 solution of acetyl-beta-methylcholine chloride had been perfused through the organ. The technique used for injecting this specimen was the same as for figure 7. In the central part of the figure a central vein, cut longitudinally, is shown entering a sublobular vein, cut perpendicularly.

Fig. 12. Same as figure 11 except a relatively great magnification was used in an effort to show the microscopic structure of a sublobular vein. Three small sluice channels may be seen piercing more or less perpendicularly through all of the tunics of the sublobular vein. The small sluice channels arise from the confluence of numerous ordinary sinusoidal capillaries in the liver lobule and drain liquid directly into the lumen of the sublobular vein. The thick, muscular wall of the sublobular vein and the small sluice channels that pierce its walls form a complex or multiple "sluice valve."

The greatest number of longitudinally arranged muscle fibers form a layer next to the tunica interna. Externally to this layer some smooth muscle fibers are arranged both circularly and longitudinally. The circular muscle fibers are found predominantly between the internal longitudinally arranged fibers next to the tunica interna and a more external layer of longitudinally arranged fibers.

Peripherally to the walls of the sublobular vein, as shown in figure 12, the liver cells are arranged in a single layer or occasionally in a double layer. Numerous "small sluice channels" (single endothelial tubes) pierce between the cells of this systematically arranged layer around the sublobular vein and drain liquid from the liver lobule directly into the lumen of this vein.

DISCUSSION. The anatomical evidence as to the nature of the mechanism responsible for regulating the outflow of blood from the liver has up to the present time been far less convincing than the physiological evidence of its existence. Bauer, Dale, Poulsson and Richards (1932) attribute the unusual behavior of the dog's liver under perfusion, and in response to chemical and nervous stimuli, to the presence of a strong muscular coat (sphincter) in the caval ends of the hepatic veins. Arey and Simonds (1920) state that the hepatic veins of the dog have "an enormous amount of smooth muscle in the wall, thus demonstrating an adequate anatomical basis for impeded vascular flow should spasm occur."

While the contraction of strategically placed non-striated muscle in the hepatic veins of the dog may serve as a satisfactory explanation for certain of the observed phenomena described by Simonds (1923), Popper (1931) and Bauer, Dale, Poulsson and Richards (1932), it does not elucidate the mechanism involved for the impounding of liquid in the liver of animals (cat, rabbit, vervet monkey, man, etc.) which do not possess unusually muscular hepatic veins.

In my own experiments the observations that physostigmin, pilocarpin and acetyl-beta-methylcholine chloride increase liver volume and decrease the outflow of liquid from the inferior vena cava strongly suggest the existence of a "resistance" (sluice valve) so located in the liver as to become effective after blood has passed through the sinusoidal capillaries. This point of view is also supported by the observations that atropine and small doses of adrenalin decrease liver volume and increase the outflow from the inferior vena cava. Corroborative evidence for the existence of such a "sluice valve" in the sublobular veins of the cat and rabbit, as well as in other animals thus far examined (raccoon, Virginia opossum, grizzly bear coati mundi, white-tailed deer and others), is found in my injected and cleared specimens (figs. 7, 8, 9, 10, 11 and 12).

Although it is generally stated that liquid enters the liver lobule by way of the "portal canals" and that liquid is drained from the liver lobule via

the central vein, my own observations show that liquid may also drain directly into the sublobular vein via small endothelial tubes (sluice channels) which arise from the confluence of many ordinary sinusoidal capillaries. As shown in some of my experiments, the outflow of liquid from the liver lobule is directly dependent upon the activity of the musculature in the walls of the sublobular veins. The size of the lumina of the small sluice channels varies greatly. This observation suggests that the small sluice channels probably open and close passively as the sublobular veins dilate and constrict. When the sublobular veins are markedly constricted, the flow of liquid through the small sluice channels is halted and the liquid is then shunted into the central veins of the liver lobules. It is both convenient and correct to speak of the central vein in each liver lobule as being a "large sluice channel". Although the lumens of the central veins also become greatly narrowed as a result of the constriction of the sublobular veins, in no instance during the course of this investigation have I ever observed their complete obliteration.

SUMMARY AND CONCLUSIONS

It is generally agreed that the "portal canals" are the gateways for blood entering the liver lobules. It is also generally agreed that the central veins drain liquid from the liver lobules into the sublobular veins. However, no mechanism has heretofore been described which adequately explains certain interesting variations of the flow of blood through the liver. Evidence for the existence of an adequate mechanism (the sluice valve) was presented in the present paper. This evidence was secured by observing the effects of certain drugs and also by examining microscopically various liver specimens after they were perfused with various drug solutions, later injected with India ink or vermilion cinnabar (in water), and then cleared. Stained preparations from the specimens were also examined.

When small dosages of adrenalin are perfused by way of the portal vein through the excised, surviving liver of the rabbit, cat and vervet monkey, the outflow of liquid through the inferior vena cava becomes markedly accelerated and the volume of the organ diminishes. Atropine produces a similar result. Large doses of adrenalin reduce hepatic outflow and diminish liver volume. On the other hand, pilocarpin, eserine and acetyl-beta-methylcholine chloride ("Mecholyl"-Merck) produce swelling of the liver and reduction of the outflow of liquid through the inferior vena cava.

The effects of pilocarpin, eserine, acetyl-beta-methylcholine chloride and small doses of adrenalin can be explained in terms of the action of a complex valve consisting of the walls of the sublobular veins and the small and large endothelial tubes (sluice channels) which enter these veins more or less perpendicularly. Pilocarpin, eserine and acetyl-beta-methylcholine chloride (in proper dosage) cause the musculature in the walls of the sub-

lobular veins to contract and thereby close (partially or totally) those portions of the endothelial tubes which are situated in the walls of these veins. The lumens of the large endothelial tubes (the central veins) also become greatly narrowed as the result of the contraction of the musculature in the walls of the sublobular veins. The "sluice valve" may be opened up widely by atropine or small doses of adrenalin. In no instance was it possible to observe anything resembling a valvular mechanism in either the walls of the portal venous radicles or in the immediate vicinity of these radicles.

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STUDIES ON TURTLE HEARTS—THE END OF SYSTOLE, THE DURATION OF THE REFRACTORY PERIOD, THE LATENT PERIOD OF EXTRASYSTOLES AND THE INFLUENCE OF HEART RATE ON AORTIC BLOOD PRESSURE

R. A. WOODBURY

From the Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta

On a summer day turtles, lying on a log in the sunshine, may have body temperatures of 30 to 35°C. In that range their metabolic and heart rates are probably elevated to values fifteen to twenty times those present at 0 to 3°C. (Clark, 1927). Accompanying the elevated metabolism, oxygen transport to tissues may be augmented by utilizing a larger amount of arterial oxygen and by increasing the blood flow. In turtles, how important is this latter mechanism and to what extent is it produced by elevation of the heart rate?

Recently, Shannon and Wiggers (1940) published aortic and ventricular pressure pulses and electrocardiograms from turtles whose hearts were artificially accelerated by "break" shocks. Nothing was done to modify the peripheral resistance or the venous return to the heart. They reported: 1st, under conditions assuring normal effective venous pressure the turtle and frog ventricle, unlike those of mammals, does not increase the minute output by an increase in rate; 2nd, the optimal heart rate is 30 to 40 beats per minute; 3rd, any further increase in rate reduces the systolic and diastolic blood pressure from which they concluded that the minute cardiac output is reduced; and 4th, the turtle ventricle is not refractory during the last third of systole.

Woodbury and Hamilton (1937) published carotid and ventricular pressure pulses of turtles with slightly higher pressures than those of Shannon and Wiggers (1940). In view of the fact that these higher pressures were obtained from turtles whose hearts were beating at nearly twice the reported "optimal rate", studies on the turtle have been continued.

Optical records of the blood pressure of nine unpithed turtles were obtained with the Hamilton "hypodermic" manometer in the manner previously described (Woodbury and Hamilton, 1937).

Acceleration of the Heart by Rythmic Electrical Stimuli. This produced

pressure changes similar to those reported by Shannon and Wiggers (1940). Small increases in the rate narrowed the aortic pulse pressure and produced small if any change in the mean pressure. Marked acceleration of the heart not only narrowed the pulse pressure but lowered the mean pressure. In some cases the diastolic as well as the systolic pressure was reduced.

These results, however, do not supply data concerning the effect of increased heart rate *by itself* upon cardiac output. When the heart is accelerated by electrical stimuli, systole is not shortened. This abnormally shortens diastole and the filling time of the ventricle. In turtles this increase in the total systolic time per minute also diverts blood into the pulmonary artery (unpublished data). Therefore, these changes in aortic blood pressure fail to supply any data concerning ventricular output.
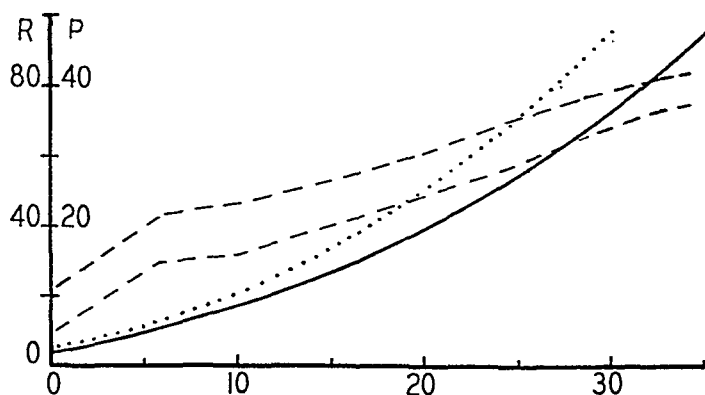
Fig. 1. Plot showing the relationship in turtles (average of six animals) between the body temperature and the heart rate (solid line) and systolic and diastolic blood pressure (broken line). The dotted line reproduces the results of Clark (1920) on the isolated frog heart. Ordinates, P = blood pressure in millimeters Hg; and R = heart rate per minute. Abscissae, body temperature.

These results also fail to determine the "optimal heart rate" under physiological conditions. Accelerating the heart artificially by means of electrical stimuli in addition to its above effects did not produce physiological changes in the vascular system or in the circulatory demands of the body. The heart, under normal regulation and coördinated to perform its normal work, may have a very different "optimal rate" from one accelerated out of all relation to the circulatory demands of the organism.

Acceleration of the Heart by Increasing the Temperature. The hearts of these same turtles were accelerated in a natural manner. This was accomplished by warming the entire turtle with water baths at temperatures up to 35°C. As shown in figure 1, the aortic blood pressure values progressively increased as the heart was accelerated. At similar heart rates the pressure values correspond very well with those previously published (Woodbury and Hamilton, 1937; Shannon and Wiggers, 1940).

Like mammals and birds (Clark, 1927), the amphibia and reptiles apparently have slower heart rates in the larger species.

At a given pressure, the rate of diastolic descent is somewhat greater at higher temperatures and at more rapid heart rates (compare the last three records of fig. 2). This is good evidence of vasodilatation and increased rate of outflow through the arterioles, if the remote possibility
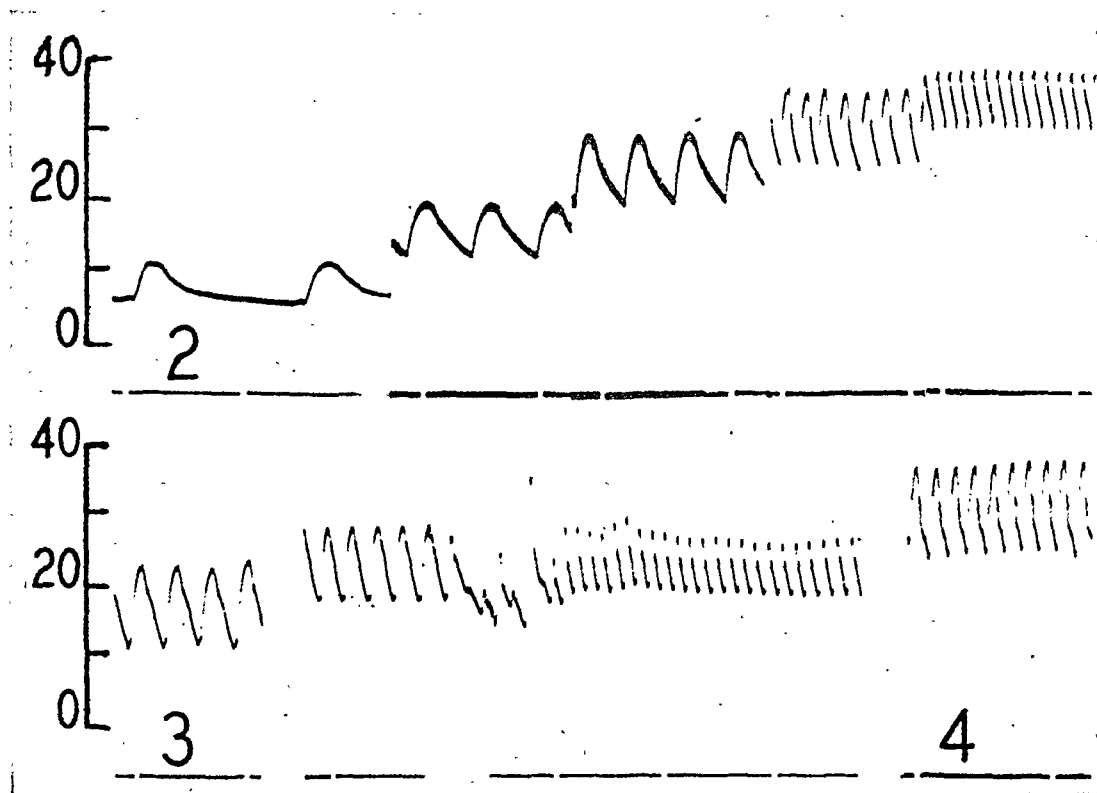
Figs. 2–4. Pressure pulses from innominate artery.. All records are from the same turtle. Blood pressure scales show millimeters Hg. Time intervals are 10 seconds.

Fig. 2. Records taken at body temperatures of approximately 0°, 8°, 10°, 18° and 28°C.

Fig. 3. The water bath was kept constant at 11°C. At break in record (elapse of 30 sec.) the heart was warmed with Ringer's solution at temperature of 30°C. At signal on base line, 38°C. Ringer's was poured upon the heart.

Fig. 4. Ten minutes later when the heart and body of turtle were nearly the same temperature (28°C.).

of constriction of the large arteries ("Windkessel") is disregarded. In spite of this vasodilatation the blood pressure did not decrease, but definitely increased (see figs. 1 and 2). Only an increase in the cardiac output into the aorta could account for the rise in blood pressure. The "optimal heart rate" for pumping blood into the aorta was not reached even at 100 beats per minute.

Warming the entire turtle affects the vascular system by warming the

body tissues and by warming the heart. Warming the body tissues increased tissue metabolism (Clark, 1927), peripheral blood flow and systemic venous return to the heart. Warming the heart increased cardiac metabolism (Clark, 1927), the speed of muscular contraction, the rate of systolic outflow and the heart rate without allowing systole to encroach abnormally upon diastole (see fig. 3).

When the heart rate is accelerated by warming the entire animal, these changes provide increased venous return, adequate time for diastolic filling, increased minute cardiac output and a rise in the aortic blood pressure values (see figs. 1 and 2).

Keeping the body temperature constant and warming only the heart should give data concerning the effect of increasing the heart rate *by itself*. This method increased the heart rate without changing the rate of diastolic descent at any given pressure (see fig. 3). This means that peripheral vasodilatation had not occurred and was not increasing the systemic venous flow. The increased speed and rate of cardiac contraction would be expected to increase the systolic and diastolic pressures until the systemic and pulmonary reservoirs are depleted. As shown in figure 3, this happened. Further increases in the rate and speed of contraction narrowed the pulse pressure, reduced systolic pressure and produced a very slight rise in the diastolic pressure.

Since there is no definite fall in mean pressure, the heart rate was not increased beyond the "optimum". Even at the rate of 48 beats per minute the circulation was maintained at a level which is certainly as great as at slower rates. The narrowed pulse pressure indicates a venous return that is not commensurate with the increased heart rate. The venous return limits the cardiac output, because when the whole body of this turtle is warmed up to a temperature which corresponds to that of the heart, the blood pressure goes up, the pulse pressure widens and circulation is increased (fig. 4). Further acceleration by warming only the heart again narrowed the pulse pressure and produced changes similar to those in figure 3. Raising or lowering the body temperature of the animal raised or lowered the heart rate value beyond which further artificial increases in the heart rate failed to increase the blood pressure.

The "optimal rate" of the turtle heart is at the arbitrary choice of the experimenter when he makes the conditions of the experiments.

THE END OF SYSTOLE, THE REFRACTORY PERIOD AND THE CHARACTERISTICS OF EXTRASYSTOLES. According to Woodsworth (1903), Marey from his classical experiments concluded that the refractory period shrank as the stimulus was strengthened, being gradually reduced to the first instants of systole and finally disappearing. Hildebrand (1877), Engelman (1895) and Woodsworth (1903) presented the definite proof that the delayed contractions caused by strong stimuli during systole were secondary

to auricular contractions arising from a spread of current. It is these latter studies that should be credited as providing the basis of our present conception that the absolutely refractory period continues to the end of systole.

Simultaneous auricular and ventricular myograms from six turtles and simultaneous pressure pulses and electrocardiograms from nine turtles supplied most of the data for this portion of the paper. Approximately two thousand strong electrical stimuli from an inductorium were applied at various times during the cardiac cycle.

Actual Time of Stimulation. At each break of the primary circuit, two stimuli really are sent to the ventricle. Either the *beginning* or the *cessation* of current flow in the secondary circuit can serve as the stimulus. Generally the beginning of flow is the effective stimulus and the cessation of flow occurs during the refractory period of the response. However, as the "break" shocks are applied earlier in the cardiac cycle, there is a period of time when the beginning of flow would be in the last portion of the refractory period of the regular contraction. The cessation of flow could then occur after the end of the refractory period and could excite the ventricle. For this reason the author has used the time of *cessation* of flow in the secondary circuit instead of the time of the "break" of the primary circuit for obtaining data concerning the refractory period.

Electrocardiograms clearly demonstrate that these two stimuli are separated by a definite but small period of time (see figs. 7, 12, 14 and 18). When using nonpolarizing electrodes and an inductorium, the time interval was 0.03 to 0.04 second. This time interval was much longer and occupied from one-fifth to one-sixth of systole in the electrocardiograms published by Shannon and Wiggers (1940). Evidently they used galvanic current. Yet, for their data concerning the refractory period, they used the beginning of current flow as the time of stimulation and disregarded the second stimulus (the cessation of current flow).

The End of Systole. Myograms and pressure pulses record the duration of systole of the ventricle as a unit or pump. On the other hand, the T wave of the electrocardiogram provides a better criterion for the ending of systole within the ventricular tissue.

At the start of an experiment, the first portion of the T wave generally corresponded fairly well with the end of mechanical systole (fig. 19). Experimental procedures, however, disturbed this relationship.

Frequently after applying a strong stimulus, the T waves of the next few regular cycles became abnormal. They started earlier in the cycle and were either heightened, flattened or inverted (figs. 5, 6, 7, 9 and 10). This early appearance and the resulting lengthening of the T wave soon persisted in most of the experiments (figs. 20, 21 and 27). This is interpreted by the author to indicate that systole was now ending prematurely
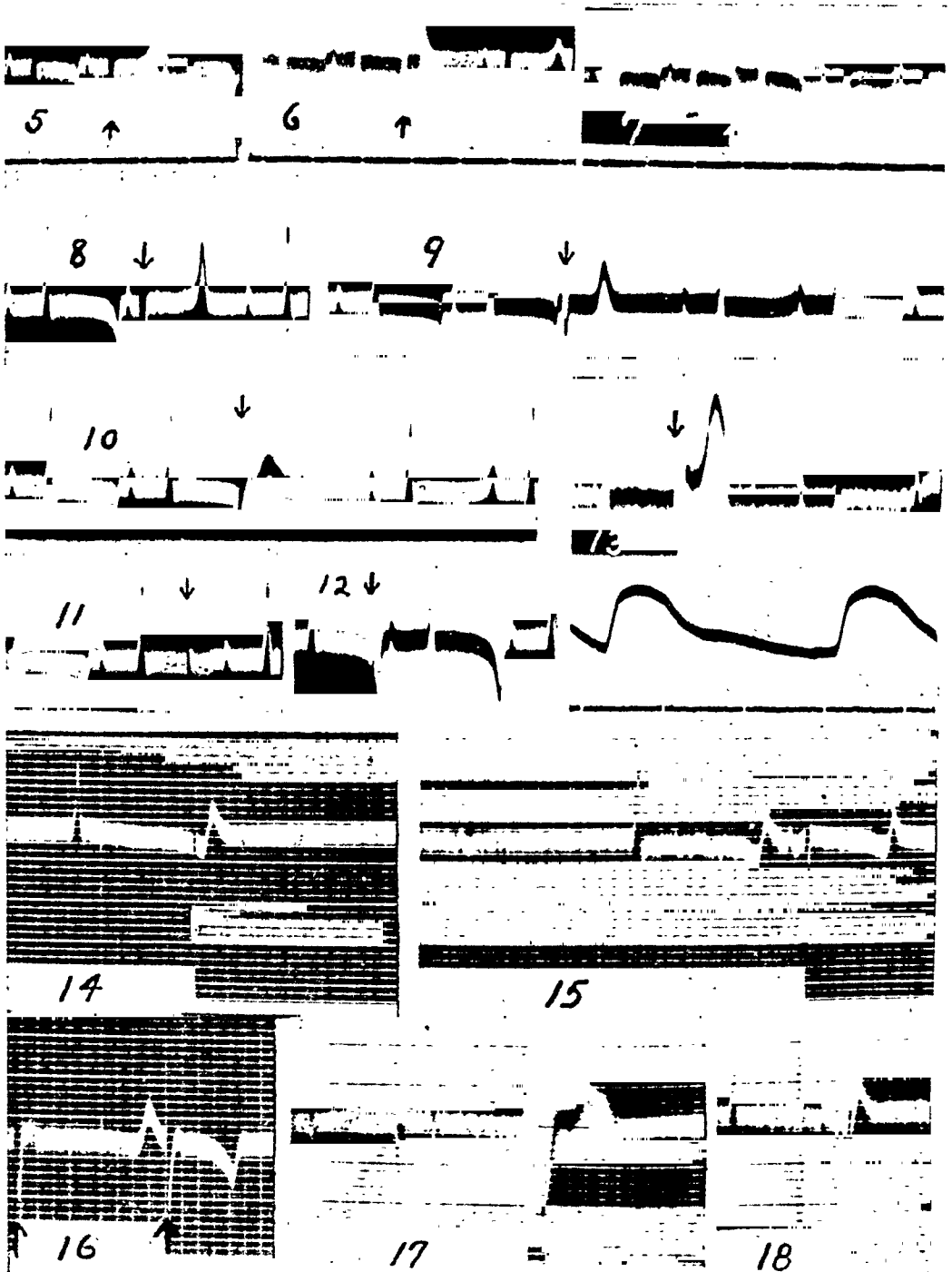
Figs. 5–13. Electrocardiograms from a turtle when stimuli (see arrows) were applied at various times during cardiac cycle. Time is shown in seconds. Time in figures 8 to 13 is the same as figure 13. The stimulating electrodes were large but were close together.

Figs. 14–18. Electrocardiogram from a turtle with complete heart block. The stimulating electrodes were large and widely separated. The arrows indicate time of stimulation.

in some small portions of the ventricle.   Even though mechanical systole was still present, diastole was starting in this small portion of the heart.
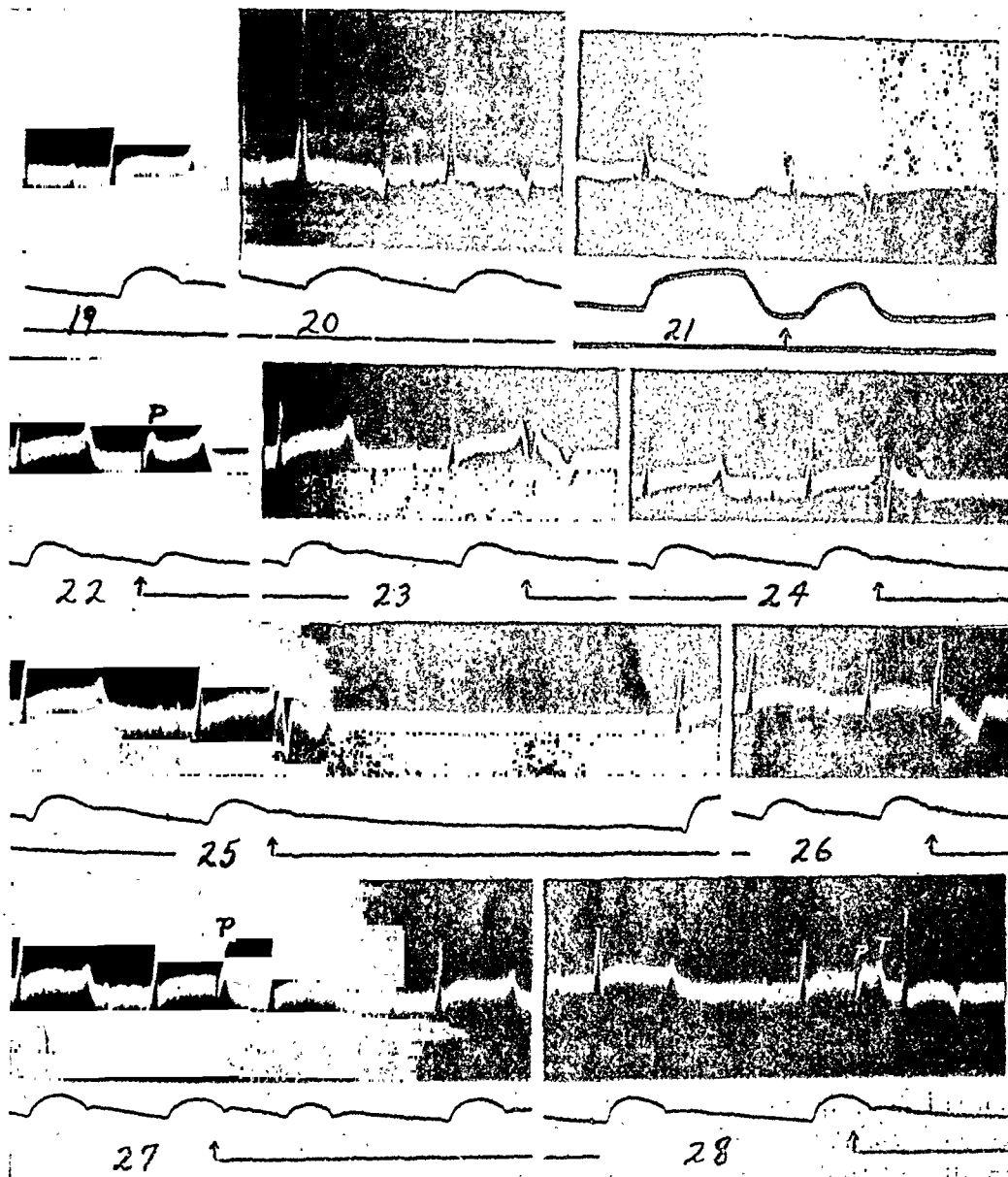
Figs. 19-21. Simultaneous electrocardiograms and aortic pressure pulses. Those in 19 and 20 were taken early in experiment, those in 21 were obtained after complete heart block.

Figs. 22-28. Simultaneous electrocardiograms and aortic pressure pulses from turtle supplying records in figure 19.   Time = 0.2 sec.   Stimulating electrodes were large and widely separated.   The arrows indicate the time of stimulation. P = P wave of extrasystole produced by current spread. T = regular T wave.

When comparing the duration of the refractory period with that of systole, the time when systole first ceases in any portion of the ventricle

should be considered as the end of systole.   Therefore, the beginning of the T wave has been used by the author as the criterion for the end of systole.

The Form of the Ventricular Complexes.   Influence of location of the stimulating electrodes.   When the stimulating electrodes were close together two waves were generally present (see figs. 5, 6, 8, 9, 10 and 13).   The spread of the wave of negativity gave rise to a modified Q R S wave.   The disappearance of the negativity produced a T wave.   Of course, the actual form of these waves varied with the location of the stimulating electrodes. When the electrodes were large and widely separated, stimulation in mid or late diastole produced a different type of electrocardiogram.   The T wave was present, but the Q R S wave was absent or very short in duration (figs. 15 and 22).   With large electrodes placed far apart the strong stimuli produced sufficient spread of current to excite simultaneously the entire ventricle.   Of course no Q R S deflection occurred for the heart was still iso-electric even though it was responding to the excitation current.   As the extrasystole ended and as the negativity of the heart disappeared, the T wave was produced.   The fact that there was a T wave is significant.   It proves that under the conditions of the experiment the duration of systole varies in different parts of the ventricle of turtles as in dogs (Wilson and Herrmann, 1921).

Effect of applying the stimuli earlier in diastole.   As in dogs (Wiggers, 1925) the extrasystoles became shorter (see figs. 5, 6, 8 and 9).   The T wave became more prolonged.   The electrical latent period did not increase which differs from the accepted observations (discussed below).

Additional and more pronounced changes were present, if the heart had been subjected to many strong electrical stimulations and if the T waves of the regular heart beats developed during mechanical systole. Excitation early in diastole (during the early part of the abnormal regular T wave) produced extrasystoles with prolonged and often bizarre Q R S and T waves.   These prolonged Q R S waves varied in form between two extremes.   At times (figs. 13, 16, 23 and 24) the wave of negativity merely spread very slowly over the ventricle and simply prolonged the Q R S wave.   At other times (figs. 17, 25 and 26) the early part of the wave was small and there was a definite delay before the wave spread over the rest of the ventricle.   This produced a bizarre, often diphasic, Q R S wave. Both types of Q R S waves indicate that only certain portions of the ventricle responded to the excitation current.   As adjoining portions finished systole and became capable of excitation, the wave of negativity spread until the whole ventricle was stimulated.

Associated with these unusual electrocardiograms, fused contractions like those reported by Cushny and Matthews (1897), delayed contractions and reciprocal ventricular contractions were recorded.   In animals with

larger hearts, are such abnormal prolongations of the T waves in the regular contractions associated with the appearance of the vulnerable period? In dogs, Wegria and Wiggers (1940) report that there is a vulnerable period during the last portion of mechanical systole (0.03–0.06 sec.) when strong stimuli will produce ventricular fibrillation. According to their published records this appears to correspond to the period of time during the development of the T wave.

The Latent Period of the Ventricular Extrasystoles. Contrary to the accepted belief, the latent period of cardiac muscle does not significantly increase as the stimuli are applied earlier in the non-refractory period. Throughout most of diastole, stimuli produced electrical responses which
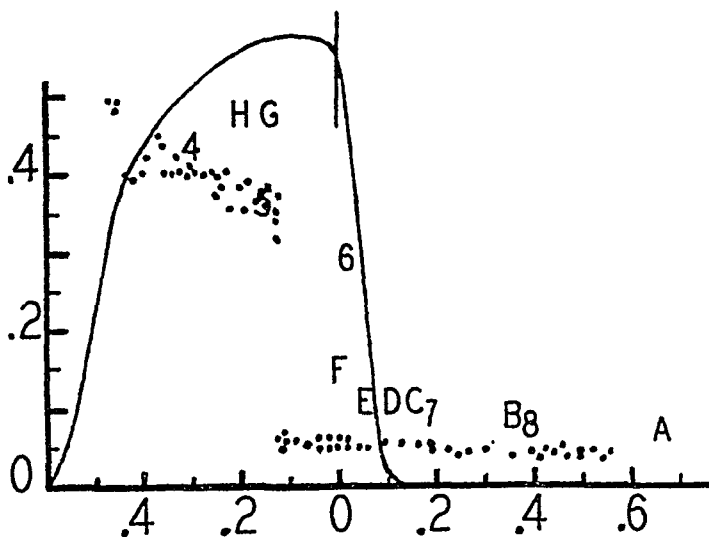
Fig. 29. Plots. Ordinates, duration of the latent periods in seconds. A ven‐tricular myogram has been sketched on the plots. Dots = data from author's records, the A-V conduction time in this animal was 0.3 sec.; letters = data from corresponding records in figure 4 of Shannon and Wiggers (1940); and numbers = data from Marey's classical myograms.

quickly spread over the ventricle (figs. 5, 6, 8, 9 and 10). Myograms and pressure pulses as well as the electrocardiograms showed very short latent periods (fig. 29). In very early diastole, stimuli also produced an *immediate* response which frequently can be demonstrated only on the electrocardio-gram. A comparison of figures 7 and 12 with 13, figures 14 and 18 with 17 and figure 27 with 24 to 26 shows that if a ventricular response occurred, the Q R S wave started immediately. The first portion may be small, but it was present and modified the regular T wave. This proves that the electrical latent period of cardiac tissue is not significantly increased as the stimuli are applied earlier in the cycle.

Since the spread of the extrasystole impulse was slow, delayed and ab-normal, myograms and pressure pulses (figs. 13 and 29) showed the accepted

lengthening of the latent period. However, this increase in the latent period of the heart as a pump, was very much less than the amount stated by Marey (Woodsworth, 1903) and Shannon and Wiggers (1940).

Reproductions of Marey's classical myograms still appear in several recent textbooks of physiology. These are said to illustrate the duration of the refractory period in the heart and the gradual increase in the latent period as the stimuli are applied earlier in the cycle (Howell, 1940; Macleod, 1938; and Starling, 1936). They actually illustrate neither of these. Assuming a heart rate of 40, time on these records has been estimated and the lengths of the latent periods have been plotted (see fig. 29). Contractions 4 and 5 appear to be the result of auricular contractions produced by a spread of current (Woodsworth, 1902-03; and Best and Taylor, 1939). The delay associated with the A-V conduction would account for their long latent period. Contraction 6 resembles those with prolonged Q R S waves where the first portion of the wave is small and the main deflection of the Q R S wave is delayed (see figs. 17, 25 and 26). In the remaining extrasystoles (7 and 8) the latent periods show no significant change in duration.

Ventricular extrasystoles originating by A-V conduction produced aortic pressure pulses (figs. 27 and 28) almost identical to some published by Shannon and Wiggers (1940). Careful measurements were made of the latent periods of these extrasystoles. These data are plotted and presented in figure 29 and fail to convince the author that their extrasystoles G and H were ventricular in origin.

The presence of a compensatory pause after extrasystoles does not serve as circumstantial proof that current spread to the auricle did not occur. The presence of this pause only proves that the current did not spread to the pacemaker, the sinus venosus in turtles. As shown in figures 7 and 27, the compensatory pause is present even though the electrocardiogram shows that current spread to the auricle does occur.

The Refractory Period. As in dogs (Wiggers, 1925) the turtle ventricle is capable of excitation during the P-R interval (see figs. 5 and 8).

Many records similar to those in figures 5 to 28 provide clear demonstrations of the continuation of the refractory period until and including the very earliest portions of the T wave. The application of the excitation current just prior to the beginning of the T wave and while the entire ventricle was still in systole (figs. 7 and 27) never produced a direct stimulation of the ventricle even though at times the excitation current originated from full strength "break" shock from an inductorium with 10 batteries in series in the primary circuit. A large proportion of the stimuli applied slightly later but definitely during the first portion of the T wave produced direct ventricular contractions (see above). However, at this time diastole has begun in some portions of the ventricle, even though the mechanical systole has not been completed.

No evidence has been observed that ventricular tissue was excitable before the end of electrical systole in some portions of the heart.

CONCLUSIONS

1. In turtles when the heart rate is modified in an artificial manner (local heat and electrical stimuli) there is lack of coördination between cardiac pumping and the vascular system.

2. When the heart is accelerated artificially, the so-called "optimal heart rate" can be raised or lowered by raising or lowering the body temperature.

3. When the heart rate increases are governed by body temperature and are coördinated with increased venous return and metabolic needs, the "optimal heart rate" is well above 70 beats per minute.

4. In the intact turtle, increasing the heart rate from 2 to 70 beats per minute by means of a natural stimulus (elevating the body temperature from 0 to 30°C.) markedly increased the systolic and diastolic pressure. This occurred in spite of vasodilatation and indicates a progressive increase in cardiac output.

5. Intact turtles possess a very effective mechanism for increasing blood flow in the systemic circulation. This is accomplished by vasodilatation, an increase in cardiac output and elevation of the blood pressure. The increased rate and speed of cardiac contraction are two important factors contributing to the increased minute cardiac output into the systemic circulation.

6. The relationship between the T wave and the end of mechanical systole differs from that generally recognized. Usually the T wave started just before the end of mechanical systole. Experimental procedures disturbed this relationship. Then the T wave would start somewhat earlier in the cycle and would be prolonged.

7. Under experimental conditions the duration of systole differs in different portions of the turtle ventricle. Prolonged repeated experimental procedures generally increased this difference.

8. Ventricular tissue is absolutely refractory to stimuli until the end of electrical systole in some portion of the ventricle.

9. As the excitation current was applied earlier in diastole, the following changes occurred.

a. Myograms and pressure pulses showed an increase in the latent period of the extrasystoles.

b. Electrocardiograms showed that the latent period of the ventricle tissue did not increase. This is contrary to the present accepted understanding.

c. The Q R S and T waves were prolonged.

d. The duration of the extrasystole decreased as previously reported.

10. Stimulation of the ventricle during the early portions of the T wave produced either fused ventricular contractions, delayed ventricular contractions or reciprocal ventricular contraction. An explanation of their production is given. In animals with larger hearts, similar conditions very likely produce ventricular fibrillation.

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RECOVERY OF FUNCTION FOLLOWING ARREST OF THE BRAIN CIRCULATION[1, 2]

HERMAN KABAT, CLARENCE DENNIS AND A. B. BAKER

From The Departments of Physiology and Neuro-Psychiatry, University of Minnesota, Minneapolis

A critical analysis of the various methods which have been employed to investigate the effects of experimental arrest of the brain circulation reveals that most of these methods are incapable of yielding accurate information (1, 2). Recently, Weinberger, Gibbon and Gibbon (1) stopped the entire circulation by clamping the pulmonary artery in cats. This method has produced quite consistent results, but it has the disadvantage of depressing the function of other vital organs such as the heart, the lungs and the kidneys.

A method has already been described which brings about cessation of cephalic blood flow in the dog (3, 4), utilizing the principle of a cervical pressure cuff. The arrest of cephalic circulation is complete and the remainder of the body is maintained in good condition by adequate oxygen supply and circulation throughout the procedure.

The results have been consistent from dog to dog when the same period of brain stasis was employed. The uniformity of the effects of this procedure in normal adult dogs of both sexes has formed the basis of the demonstration of the greater resistance of young animals (5) and the decreased resistance of pregnant or lactating females (6) to arrest of the brain circulation.

RESULTS. *A. Disappearance of responses during acute arrest of the brain circulation.* Complete arrest of the brain circulation was produced in 31 adult dogs for periods ranging from 2–11¼ minutes. The corneal reflex as a rule disappeared in 10 to 20 seconds but in several experiments could no longer be elicited 5 seconds after cervical compression. Respiratory movements ceased 15 to 20 seconds after compression in most instances and never persisted longer than 30 seconds. Urination frequently

occurred during the first minute. The pulse was strong and rapid during the first few minutes but became weaker as the stasis was continued. After 1½ to 2 minutes of stasis, the animal relaxed, no spontaneous movements of any type were observed and profound spinal shock became evident.

B. Behavior of surviving animals. The results were constant from dog to dog and there was no evidence that the effects were influenced by the sex, weight, breed or age of the normal adult animal. All animals which survived a period of arrest of brain circulation of 6 minutes or less recovered function apparently completely, while animals which were subjected to periods of stasis of 8 minutes or longer invariably suffered permanent damage to the brain.

The course of recovery following various periods of brain stasis is essentially similar except for differences in time relations dependent on the duration of the stasis. The course of recovery can be divided conveniently into the following periods: 1, period of early return of function; 2, period of hyperactive coma; 3, period of quiescent coma; 4, period of apathy and severe ataxia; 5, period of residual ataxia; 6, recovery. All of the experimental animals went through the first three periods but only animals surviving 6 minutes or less of brain stasis showed the last three periods of recovery.

1. Period of early return of function. Throughout this period, the dog remained in coma and was flaccid. The average figures for return of function of respiration and the wink reflex are illustrated in figure 1. The recovery time of the corneal reflex was four times that of the respiratory center. Recovery time increased sharply when the arrest of circulation exceeded the critical period of 7 minutes.

The threshold of spinal reflexes was initially high and fell progressively, indicating a gradually receding spinal shock. At first, stimulation was effective in eliciting only the homolateral flexion reflex and only some time later was it possible to produce a crossed extension reflex. In one experiment, after an 8-minute period of brain stasis, the crossed extension reflex first appeared 50 minutes after restoration of blood flow in the brain.

Shivering was a frequent occurrence during the early hours after the cephalic stasis, even though the rectal temperature was normal or elevated. The rectal temperature was, as a rule, only slightly above normal in the first hours after restoration of blood flow, rising to 102 to 103°F., whereas the normal rectal temperature ranged from 101 to 102°F.

In one dog, the circulation to the brain was stopped for 11 minutes and 15 seconds and the animal survived for only 2 hours. This experiment is of interest because the rectal temperature in this animal rose to 110°F., indicating acute failure of the heat-regulating mechanisms. The animal showed gasping respiration during this period and the corneal reflex

never returned. The pupils gradually constricted and at 25 minutes after restoration of blood flow, became 1 mm. in diameter and remained so until death. The dog regained the flexion reflex but only a weak crossed-extension reflex.

2. *Period of hyperactive coma.* A characteristic phenomenon which appeared some time after resuscitation and persisted for several hours was the occurrence of rapid running movements of all limbs, often accompanied by salivation and vocalization. These movements, which were well-
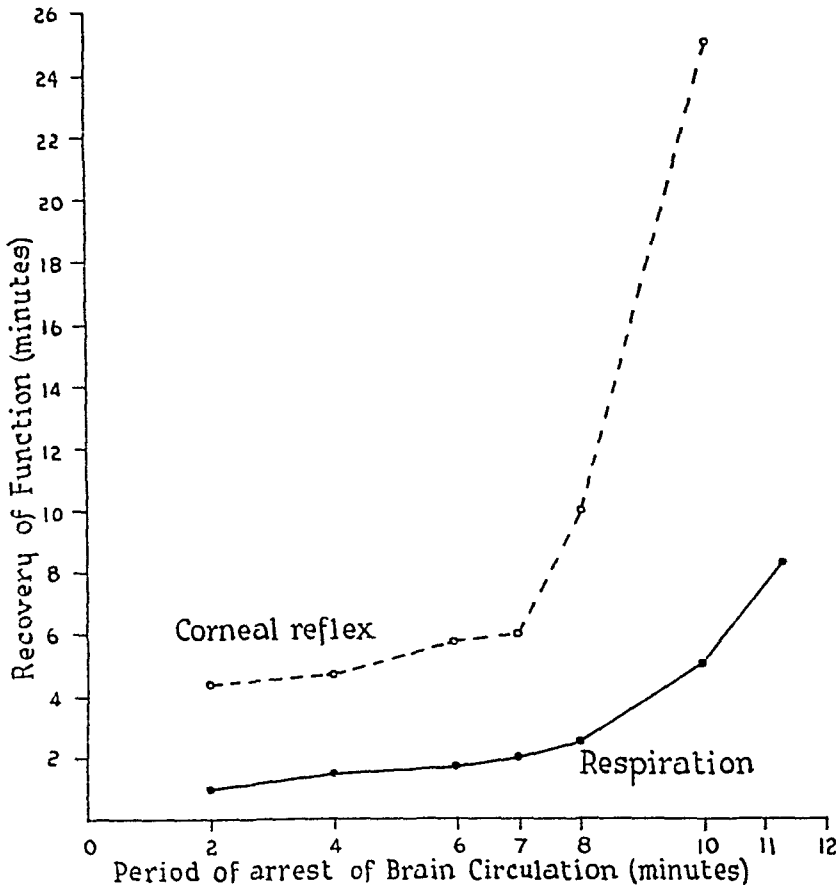
Fig. 1. Recovery of function of the respiratory center and of the corneal reflex following arrest of the brain circulation in adult dogs. The points represent average values.

coördinated and rhythmic, were carried out with the dog lying unconscious on his side. The movements were occasionally interrupted spontaneously. During a quiescent period, tactile or visual stimuli caused a resumption of the running movements. There was hyperirritability of knee jerks.

Early in the period of hyperactive coma, some extensor rigidity was observed. The head usually showed opisthotonus and the jaws were closed rigidly. In intervals between running movements, moderate extensor rigidity was evident in the limbs, predominantly in the forelimbs.

Even in cases in which the extensor rigidity was rather marked, however, it was insufficient to support the weight of the body. Labyrinthine righting reflexes were absent. Auditory stimuli were ineffective and visual reflexes included constriction of the pupils and lid movements.

No convulsions were observed following *complete* arrest of the brain circulation. On the other hand, in one case in which the blood flow through the brain was stopped *incompletely* for 15 minutes, gradually increasing convulsions were seen, culminating in status epilepticus.[3]

3. Period of quiescent coma. Coma persisted for a long time even after brief arrest of blood flow in the brain (table 1): after 2 minutes of stasis for 12 to 18 hours; after 6 minutes, for 24 hours or longer; after 8 or more minutes, coma was permanent.

The characteristics of quiescent coma were observed in all of the experimental animals, but were most easily studied in the dogs subjected to the longer periods of brain stasis. The animal showed no spontaneous movements and was unconscious. Auditory and olfactory reflexes were absent while pupillary and lid reflexes to light were present. The threshold of response to noxious stimulation was greatly elevated, and there was no localization of the stimulus, no emotional reaction or integrated purposive response. The homolateral rhythmic scratch reflex of the hind leg could be elicited but the movement was poorly directed.

The limbs and tail were flexed, the neck was rigid, the head moderately extended with masseter muscle spasm. There was resistance to passive extension of the flexed limbs and tail. When the limbs or tail were passively extended, they returned slowly to the original flexed position. Rigidity was present in the proximal but absent in the distal joints of all limbs. With the dog lying on his side, the flexor rigidity was equal on the two sides of the body. When the animal was supported in space in various positions or the head was rotated, he maintained a constant posture, but typical catatonia did not appear. Pressure on the foot pad failed to evoke reflex extension of the limb. Vestibular and righting reflexes were absent. In some experiments with longer periods of brain stasis, a persistent spontaneous rapid vertical nystagmus was evident for several days. The knee jerks were hyperactive.

Despite the persistent coma, complex reactions associated with feeding and elimination, which were absent during earlier periods of recovery, were regained during this period. When the head was supported and the mouth held in milk, the animal lapped mechanically and could be fed in this way.

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[3] In this dog, periodic spasmodic twitching of the hind limbs and mouth began 27 minutes after restoration of blood flow. The convulsions developed progressively until 1½ hours after the stasis when the dog showed violent spasms occurring in very rapid succession. The convulsions were clonic in character, with simultaneous spasms of the limbs, tail, ears and mouth. Even the pupils participated in the convulsions with a synchronous marked hippus.

TABLE 1

Effects of various periods of arrest of the brain circulation in normal adult dogs

DOG	SEX	PERIOD OF BRAIN STASIS	SURVIVAL	DURATION OF COMA	POSTURE	PERSISTENCE OF APATHY	PERSISTENCE OF ATAXIA	END RESULT AND COMMENT
		min-utes		*hours*		*days*	*days*	
1	M	2	4 months	12–18	Can stand after 18 hours	2	7	Apparently normal
2	F	2	4 months	12–18	Can stand after 18 hours	2	5	Apparently normal
3	F	4	19 days	18	Can stand after 18 hours	4	19	Apparently normal except for very slight ataxia
4	M	4	3 months	18	Can sit up on 2nd day. Can stand on 3rd day	4	21	Apparently normal
5	M	4	4 months	24	Can sit up after 24 hours. Can stand after 36 hours	3	18	Apparently normal
6	F	6	Still alive (1 year)	40	Can sit up after 24 hours. Can stand after 40 hours	4	40	Apparently normal
7	M	6	3 months	24	Can sit up after 24 hours. Can stand after 48 hours	3	20	Apparently normal
8	F	6	3 months	24	Can sit up after 24 hours. Can stand after 48 hours	3	20	Apparently normal. Littermate of 7, identical course of recovery
9	F	8	3 days	Comatose throughout	Lies on side, opisthotonus. No righting reflexes			No recovery from coma
10	F	8	4 days	Comatose throughout	Lies on side. No righting reflexes			No recovery from coma
11	M	8	5 days	Comatose throughout	Lies on side. No righting reflexes			No recovery from coma
12	M	8	2 days	Comatose throughout	Lies on side. No righting reflexes			No recovery from coma
13	M	8	2½ months	Comatose throughout	Can sit up after a week. Never stands			Permanent defects: (see text)
14	F	10	6 days	Comatose throughout	No righting reflexes			No recovery from coma. Drinks milk when mouth is placed in it. Complex responses in elimination
15	F	11¼	2 hours	Comatose	Lies on side. No righting reflexes			Continues to gasp until death. Rectal temperature rises to 110°F. Pin point pupils. Spinal shock

Lapping was rapid and vigorous at first, but after ingestion of some quantity of milk, gradually ceased, after which the head was withdrawn. The

comatose dog lifted the hind leg in urination. Elevation of the tail, flexion of the thighs and pelvis, running movements of the hind legs, vocalization and sometimes rhythmic chewing and lapping accompanied defecation.

Respiration was usually slow and deep, and there was no sign of a Cheyne-Stokes rhythm. There was a definite bradycardia, with a pulse rate as low as 63 per minute and a marked respiratory arrhythmia of the heart. Body temperature was normal but shivering occurred at environmental temperatures of 80°F.

4. Period of apathy and severe ataxia. Dogs which survived arrest of brain blood flow for 8 minutes or longer never recovered to this stage. In animals which were subjected to brain stasis of 6 minutes or less, a period of dullness and apathy intervened between the period of coma and recovery of function of the higher centers (table 1).

The earliest sign of recovery from coma was the restoration of vestibular function and righting reflexes, which enabled the animal to turn from his side to his abdomen, although still unable to stand or walk. Flexor rigidity was rapidly disappearing. In sitting up, he frequently stepped on the dorsum of the forefoot. The forelimbs appeared to be much stronger and more active than the hindlimbs, and were used in crawling on the abdomen.

During this early period of returning brain function, spontaneous movement was at a minimum, consisting of cleaning and licking, sitting up, turning the head and attempts to crawl. As earlier, milk was lapped. If now the food was moved beyond reach, the hungry dog extended his head toward it but made no attempt to crawl.

The emotional behavior of the animal was abnormal. Visual or auditory stimuli elicited a type of "sham rage". The presence of this response was a quickly passing phase of recovery, since less than a day later it was impossible to evoke the pattern.

The next stage of recovery was characterized by return of the ability to stand and walk and increased spontaneous activity. At first the dog stood on a broad base, constantly shifting his position and showing severe ataxia, which was similar to that seen in cerebellar dysfunction in man. There was at this stage no evident rigidity, paralysis or defect in conscious proprioception. The tendency to step on the dorsum of the forefoot persisted.

While cerebral function had improved by this time, there were still evidences of depression of the higher centers. Although the dog now recognized food and fed himself, there was no response to other animals. He was unable to perform previously learned tricks and was still lethargic.

5. Period of residual ataxia. At this stage, the behavior of the animal could not be distinguished from the normal by simple observation in the

laboratory. He regained the ability to perform tricks without relearning. The dog fell infrequently now, no longer stepping on the dorsum of the forefoot. He still suffered from an ataxic gait, this being the only residual deficiency. Coördination of movement gradually improved and later a deficiency was evident only when the animal was excited and very active or when the floor was wet.

6. Recovery. All dogs surviving periods of arrest of the brain circulation of 6 minutes or less eventually recovered completely, as far as we could determine without the employment of special technique for study of the higher cerebral functions. Such animals have been kept under observation in the laboratory for several months, during which time they have behaved normally.

C. Permanent brain damage resulting from 8 minutes of arrest of blood flow. One dog which was subjected to arrest of the brain circulation for 8 minutes was kept alive for $2\frac{1}{2}$ months, during which time only slight recovery of brain function was observed (dog 13, table 1). He recovered to a stage between quiescent coma and apathy. Auditory and visual reflexes, sensation, feeding reactions, and cardiac rhythm remained as described in the section above on quiescent coma. However, this animal regained some vestibular and righting reflexes. Spontaneous movements were rare and consisted of sitting up, turning the head, licking chops and licking the fur, the latter being insufficient to keep the body clean. He regained no power of locomotion or vocalization, and emotional responses were not elicited. When the animal was supported in the upright horizontal position with the limbs free, extensor spastic rigidity became apparent. This spasticity was more marked in the forelimbs than in the hindlimbs, while there was no extensor rigidity of the neck, and the tail was markedly flexed, curled under the abdomen and rigid. Passive rotation of the head usually failed to alter the posture of the limbs. The placing reaction to touching the dorsum of the foot to the edge of the table was absent in the forelimbs but present in the hindlimbs. When the dog was placed on his side or back, a flexor rigidity similar to that seen in quiescent coma became evident.

DISCUSSION. The marked functional depression of the cerebellum by arrest of the brain circulation, as indicated by the persistence of ataxia long after the restoration of cerebral function, suggests that the cerebellar neurons may be the most sensitive to anoxia. This view is supported by the fact that the cerebellar cortex shows the greatest oxygen consumption (7), and that action potentials disappear first in this region during brain anemia (8). Observations in humans subjected to anoxia (9) and carbon monoxide (10) also confirm the greater susceptibility of the cerebellum. In patients resuscitated after hanging (11), cerebellar symptoms persisted after recovery of cerebral function. Microscopic studies of the brain in

dogs (12) and cats (13) reveal that the Purkinje cells of the cerebellar cortex are most susceptible to arrest of blood flow. Since the Purkinje cell is an essential link in the neuron chain of cerebellar activity, any damage to these neurons is immediately reflected in loss of function.

Many of the phenomena observed following brain stasis have been described in decorticate animals (14): Stepping on the dorsum of the forefoot, infrequent restricted spontaneous movements, loss of placing reactions, inadequate cleaning responses, hyperirritability of the shivering mechanism (15), retention of taste and of complex responses of feeding and elimination. The extensor rigidity which appeared when the dog was supported in the upright position with the legs free is similar to "decorticate rigidity" (14, 16), although spasticity was not observed in the dorsal decubitus in our experiments. The blindness and anosmia were probably cortical in origin. In contrast to decorticate preparations, our animals lacked auditory reflexes.

The marked flexor statue-like rigidity observed during the period of quiescent coma is suggestive of altered function of the basal ganglia. This rigidity has many of the characteristics of basal ganglion disease in the human, being "soft", flexor in type, showing resistance that is equal throughout the passive manipulation with no lengthening or shortening reactions, involving only the more proximal joints of the limb and being uninfluenced by rotation of the head or changes in the position of the head in space. When the limb is passively extended, it returns only slowly to the original flexed position. In comatose patients resuscitated after hanging, flexor rigidity attributed to loss of function of the globus pallidus has been observed (11, 17). It is well known that the basal ganglia are very susceptible to anoxia in man, as seen in carbon monoxide poisoning and arteriosclerosis.

A number of other findings in dogs following brain stasis are referable to dysfunction of the brain stem: loss of auditory reflexes, loss of emotional reactions and vocalization, loss of the ability to stand and walk, loss of vestibular and righting reflexes. It is of interest to note that dogs sacrificed soon after acute carbon monoxide asphyxia show histological changes in the vestibular nuclei while animals surviving the acute asphyxia for several months have normal vestibular nuclei (18). Another characteristic finding, the bradycardia and respiratory arrhythmia observed in comatose dogs, can perhaps be explained as a release of the vagus center from higher inhibition (3).

The period of hyperactive coma, characterized by spontaneous coördinated running movements and vocalization, has been observed following brain anemia in cats (1) and dogs (19). In man, running movements, inarticulate vocalization and reflex hyperexcitability have been described in the early period of recovery from strangulation (11, 20) and from cardiac

arrest (21). Similar running movements occur during recovery from barbiturate anaesthesia.

Various workers (1, 2, 22) have reported the occurrence of epileptiform convulsions, sometimes developing into fatal status epilepticus, in cats following arrest of the brain circulation. On the other hand, in our own experiments and those of others with dogs (19), as well as in clinical studies of arrest of the circulation to the human brain (11, 20, 21), convulsions did not occur following restoration of blood flow. In the single instance in our experiments in which epileptiform convulsions appeared, the brain stasis had been incomplete. While arrest of blood flow was incomplete in some experiments with cats (2, 22), this was apparently not the case in others (1) and one must resort to a species difference to explain the discrepancy. This problem has been discussed from the clinical point of view by Cobb (23), who comes to the conclusion that partial anemia is much more likely to produce convulsions than complete anemia of brain tissue.

The statement which was made previously that dogs which survived complete arrest of the cephalic circulation of 6 minutes' duration recovered apparently completely must be qualified, since the examination of cerebral function was limited to observation in the laboratory and tests of ability to retain simple tricks learned previously and to learn such responses anew. The possibility of permanent disturbance of the higher functions of the cerebral cortex cannot be disregarded, since Andreyev (24) has demonstrated, by the method of conditioned reflexes, deficiencies in higher functions in dogs which appeared normal in ordinary examination following brain anemia.

The similarity of the course and detailed findings in patients recovering from acute strangulation (11, 20) and of the effects of arrest of the brain circulation in our experimental animals is very striking. These patients show the period of hyperactive coma with running movements and inarticulate vocalization, the early extensor rigidity and opisthotonus, the later flexor rigidity and extrapyramidal signs, the return of cerebral function with disorientation and clouding of consciousness, the late cerebellar symptoms and the eventual complete recovery. This similarity leads one to hope that the investigation of the effects of arrest of the brain circulation in animals may prove to be of value in the understanding of circulatory disorders in the human brain.

SUMMARY AND CONCLUSIONS

1. By means of a new technique, sudden complete arrest of blood flow in the brain has been produced in dogs.

2. The course of recovery is qualitatively similar but differs quantitatively in different dogs depending on the duration of the arrest of the cephalic circulation. The course of recovery may be conveniently divided

into six stages: a, period of early return of function; b, period of hyperactive coma; c, period of quiescent coma; d, period of apathy and severe ataxia; e, period of residual ataxia; f, recovery.

3. Complete arrest of the brain circulation results in disappearance of the corneal reflex in 10 seconds and of respiratory function in 20 to 30 seconds.

4. Arrest of the cephalic circulation causes spinal shock, which passes off gradually after blood flow is restored.

5. Arrest of the brain circulation for 6 minutes or less results in apparently complete recovery of function, while brain stasis for 8 minutes or longer results in permanent severe damage to the brain. The effects of cephalic stasis are the same for each period of stasis investigated regardless of the sex, weight or breed of the experimental animals.

6. Relatively soon after restoration of function of the vital centers, a period of hyperactivity and reflex hyperirritability ensues which persists for several hours. This period is characterized by vigorous, rapid, coordinated running movements of all 4 limbs carried out with the animal lying on his side in coma. No epileptiform convulsions have ever been observed following complete arrest of cephalic blood flow.

7. Coma may persist for as long as 24 hours after only 4 minutes of arrest of the brain circulation. The coma is characterized by loss of auditory reflexes, vestibular and other righting reflexes in addition to loss of function of the cerebral cortex. Early in coma a moderate spasticity is evident, while later a flexor rigidity suggestive of involvement of the basal ganglia becomes apparent.

8. Following coma, there is a transition period lasting several days which is characterized by gradually improving function of the cerebral cortex and of the righting mechanisms, as well as by severe ataxia of cerebellar type.

9. In dogs which recover consciousness, the most persistent neurological dysfunction is ataxia. This gradually disappears, leaving an animal which cannot be distinguished by our methods of examination from the normal.

10. The permanently defective brain function produced by 8 minutes of arrest of brain blood flow consists of loss of function of the cerebral cortex, loss of auditory reflexes, of the ability to stand and walk, of emotional reactions and vocalization, as well as dysfunction suggestive of striatal involvement.

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A QUANTITATIVE METHOD FOR THE MEASUREMENT OF THE RATE OF WATER LOSS FROM SMALL AREAS, WITH RESULTS FOR FINGER TIP, TOE TIP AND POSTERO-SUPERIOR PORTION OF THE PINNA OF NORMAL RESTING ADULTS

CHARLES NEUMANN, ALFRED E. COHN AND GEORGE E. BURCH[1]

From the Hospital of the Rockefeller Institute for Medical Research, New York

Most of the methods previously described for the measurement of the rate of sweating from small areas of the human skin have been qualitative (1, 2, 3, 4, 5). A number of quantitative methods have been advocated (5, 6, 7, 8). We found that those that involve the use of an absorbent material, such as filter paper (8) or cloth (6) are inaccurate and non-reproducible. Kuno (5) studied the rate of sweating by using U-tubes filled with calcium chloride to trap the moisture picked up by dry air that had been allowed to flow over a small area of skin. In attempts to use this method it was found that the removal of moisture by calcium chloride U-tubes was not complete at the rate of flow of air necessary to remove the moisture from the skin. Even 1500 grams of finely divided calcium chloride enclosed in a glass tube four feet long did not adequately dry such a stream of air or oxygen. Greuer and Peukert (7) described a method for ascertaining the amount of moisture lost from human skin by measuring variations in the resistance of a semi-conductor. In their method a known area of skin was covered with a shallow chamber roofed by a sodium chloride crystal. The rate of change in resistance of the crystal produced by the water evaporating from the skin and being deposited upon its surface was used as an index of the rate of the elimination of water. Lack of satisfactory standardization of the method made it inadvisable to use it in its present form. A more complete review of the various procedures that have been employed can be found in the publications of Kuno (5), McSwiney (6) and Greuer and Peukert (7). Because of the inadequacy of these methods it became desirable to develop a more accurate procedure. This report concerns itself with a description of, and the results obtained with, the method evolved.

MATERIALS AND METHOD. The apparatus can be divided into three parts: 1, metal cups enclosing the part studied; 2, aluminum coils for the collection of water; 3, a system for providing a stream of dry oxygen.

[1] Commonwealth Fund Fellow.

1. The chambers for enclosing the finger tips and toe tips were constructed of brass sheeting (0.003 inch thick), cut, shaped and soldered together to form cylinders (diameter about 3 cm.; height, about 4 cm.) (fig. 1 A). The chambers were made of metal instead of cellulose acetate since this substance permits a slow diffusion of water. An opening was made in one end (the proximal end) of the cylinder for the entrance of the part to be studied. Four radial brass tubes (inlets) with an inside diameter of approximately 2 mm. were soldered into the circular wall of the cylinder near the proximal end. A fifth brass tube (outlet) with an inside diameter of approximately 3.5 cm. was soldered to the center of the distal end of the cylinder. A short piece of flexible rubber tubing was fitted over the proximal end of the cylinder. To make an air-tight seal this tubing was
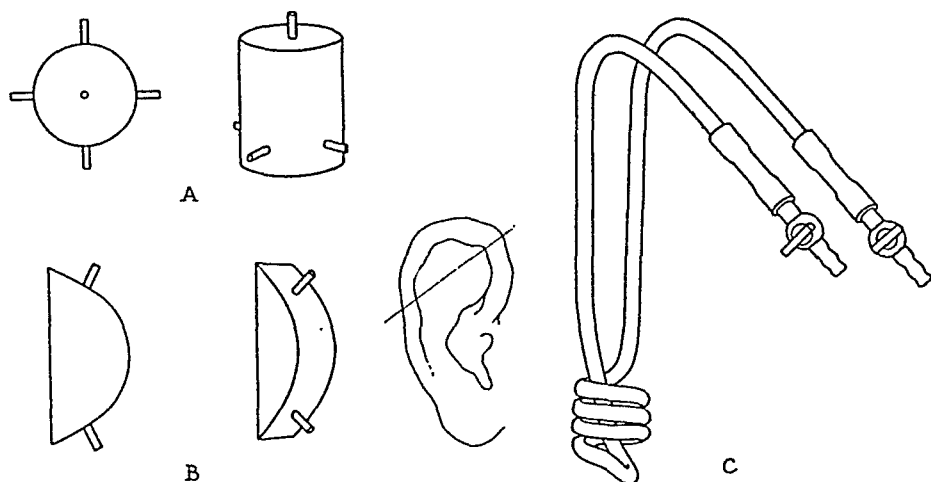
Fig. 1. A—Brass cylinder for enclosing finger tip or toe tip. B—Brass hemi-cylinder for enclosing postero-superior portion of pinna. C—Aluminum coil for collecting water.

chosen to fit closely to, but without constricting, the part inserted for study.

The chamber for enclosing the postero-superior portion of the pinna was also constructed of brass sheeting but was a hemi-cylinder (height about 1.5 cm.; radius about 2.5 cm.) (fig. 1 B). A wide more or less crescent-shaped opening was cut into the flat surface (base) and closed by a rubber membrane. In this an opening was cut, so shaped as to conform accurately to the shape of the pinna lying in the opening. A brass tube (inside diameter approximately 3 mm.) was soldered to each end of the curved wall. One tube served as an afferent and the other as an efferent.

2. A number of aluminum coils (fig. 1 C) for the collection of water was constructed of approximately 1 meter of aluminum tubing (outside diameter 4.8 mm.; inside diameter 3.2 mm.). Each end of the coils was guarded by a metal stopcock. The coils were made to weigh 50 grams to facilitate successive weighings.

3. A stream of oxygen flowing from a tank passed through rubber tubing to an aluminum coil (fig. 2). The coil was placed in a thermos bottle containing a freezing mixture of ethyl alcohol and CO_2 snow. In this coil the oxygen was dried. From the coil it passed in an aluminum tube at least 12 feet long and a four-way distributor into the four inlets of a brass cylinder. From the brass chamber, the oxygen, now carrying sweat, proceeded by rubber and aluminum tubing to a second aluminum coil. This coil was also placed in a thermos bottle containing a freezing mixture of ethyl alcohol and CO_2 snow. By means of a system of stopcocks and
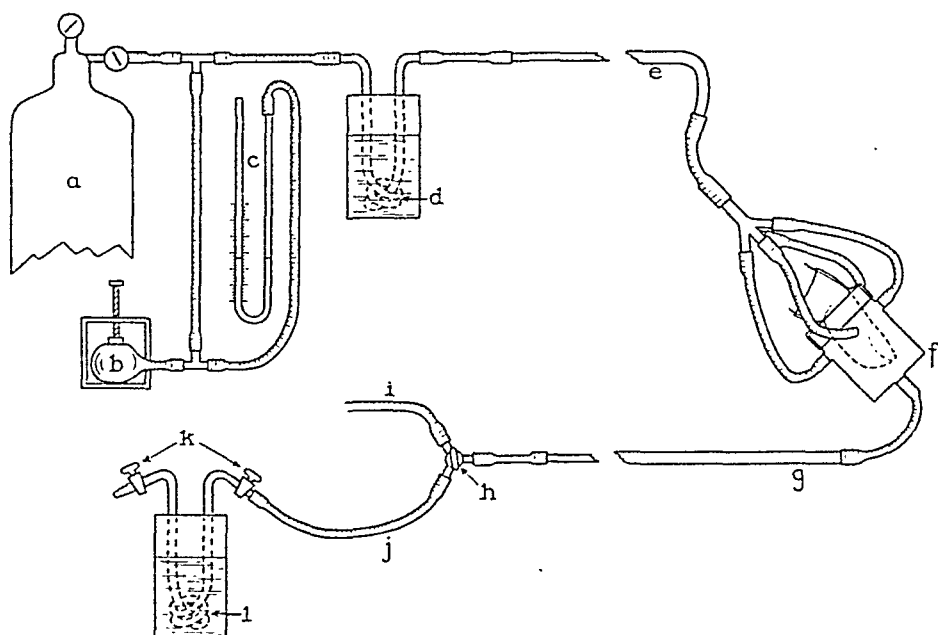
Fig. 2. A complete diagram of the apparatus. *a*, oxygen supply; *b*, pressure bulb controlled by screw clamp; *c*, water manometer; *d*, aluminum coil for drying the oxygen; *e*, aluminum tubing conducting dry oxygen to part; *f*, metal cylinder enclosing part; *g*, aluminum tubing conducting water laden oxygen from part; *h*, 3-way stopcock permitting distribution of oxygen through rubber tubing, *i* or *j*, to alternate water collecting coils; *l*, aluminum water collecting coil with stopcocks, *k*.

rubber tubing it was possible to change the flow to a number of aluminum coils in succession, in order to collect sweat for varying periods of time.

To ascertain the accuracy of the method a brass chamber containing a few drops of water was substituted for the usual cylinder. The air in the chamber was displaced with oxygen and its initial weight obtained. Dry oxygen was passed through the rubber and aluminum tubing for 15 minutes to insure dryness of the system. This oxygen was then permitted to flow through the brass chamber and a weighed aluminum coil immersed in the freezing solution. The water vapor carried from the brass chamber was

deposited in the aluminum coil and the oxygen, having deposited its water, was allowed to escape. After a few minutes the brass chamber was removed. The lengths of tubing which had been connected to the inlet and outlet tubes of the chamber were connected to each other and dried with dry oxygen for 15 minutes. The stopcocks of the aluminum coil were then closed to prevent the escape of water when the coil returned to room

TABLE 1

Data showing the results of the standardization of the method

SAMPLE NUMBER	WATER INTRODUCED INTO THE SYSTEM	WATER COLLECTED BY APPARATUS	DIFFERENCE	ERROR	DIFFERENCE AFTER CORRECTION OF 0.4 MGM.	ERROR AFTER CORRECTION
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
1	69.6	71.1	+1.5	+2.15	+1.9	+2.7
2	49.9	49.8	−0.1	−0.20	+0.3	+0.6
3	47.4	46.1	−1.3	−2.74	−0.8	−1.7
4	31.8	31.1	−0.7	−2.20	−0.3	−0.9
5	31.2	30.6	−0.6	−1.92	−0.2	−0.6
6	28.0	27.3	−0.7	−2.50	−0.3	−1.1
7	21.4	21.1	−0.3	−1.40	+0.1	+0.5
8	20.4	20.6	+0.2	+0.98	+0.6	+2.9
9	20.3	19.9	−0.4	−1.97	0.0	0.0
10	17.3	17.9	+0.6	+3.46	+0.9	+5.2
11	15.6	15.5	−0.1	−0.64	+0.3	+1.9
12	14.2	13.5	−0.7	−4.93	−0.3	−2.1
13	14.0	13.2	−0.8	−5.71	−0.4	−2.9
14	12.1	11.5	−0.6	−4.95	−0.2	−1.6
15	12.0	12.1	+0.1	+0.83	+0.5	+4.2
16	12.0	11.4	−0.6	−5.00	−0.2	−1.6
17	11.7	10.8	−0.9	−7.69	−0.5	−4.3
18	7.5	6.8	−0.7	−9.30	−0.3	−4.0
19	7.2	6.7	−0.5	−6.99	−0.1	−1.4
20	5.3	5.1	−0.2	−3.70	+0.2	+3.8
21	4.3	3.8	−0.5	−1.16	−0.1	−2.3
22	3.6	2.9	−0.7	−19.40	−0.3	−8.3
23	3.4	2.7	−0.7	−20.50	−0.3	−8.8
24	3.2	2.8	−0.4	−12.50	0.0	0.0
25	2.6	2.2	−0.4	−15.30	0.0	0.0
Mean.......	18.6	18.2	−0.4			2.6

temperature. The brass chamber and the aluminum coil were both weighed a second time to learn the amount of water which had been lost from the former and the amount gained by the latter. This procedure was repeated for 25 separate measurements (table 1).

The mean loss of water from the brass chambers was 18.6 mgm. and the mean amount of water picked up in the aluminum collecting coils was 18.2

mgm., an error of −0.4 mgm. or −2.2 per cent (table 1). The variation from this mean error was small. Such an error, insignificant for large amounts, became increasingly important as the amount of water deposited decreased. Since the error was about 0.4 mgm. and since we were not in position to learn its source, we corrected our results arbitrarily by the addition of this amount. The correction is applicable in the 15 subjects.

To measure the rate of perspiration, fingers, toes or ears were sealed in their brass cylinders with rubber cement. Since three parts were studied simultaneously, three separate streams of dry oxygen were provided. Leaks were detected by the use of a water manometer. The oxygen flow was adjusted to 300 to 500 cc. per minute in each chamber, pressure not exceeding 3.5 cm. of water. For the first 30 minutes, oxygen being used to dry the systems was allowed to escape. To collect and measure the water eliminated, the oxygen was made to pass through the aluminum coils immersed in the freezing mixture. After a certain time the flow having passed through one group of aluminum coils was directed through a second group. Flow through a succession of coils was carried out for 15 minute intervals for a total of 60 to 90 minutes.

Certain precautions in weighing the coils were taken: 1. To insure uniform dryness before use the inside of the coils was dried by passing room air through them and the outside by blowing room air over them. The coils were then filled with dry oxygen at atmospheric pressure before being weighed. 2. After the collection of the water, the oxygen in the coils having attained room temperature was brought to atmospheric pressure by opening one of the stopcocks momentarily to allow the escape of the excess of oxygen.

The flow of the dry oxygen through the brass chambers did not affect their temperature materially as measured by thermocouples placed within the chambers.

Because of variations in the size of the parts studied, measurements of the surface area of the finger tip, toe tip and postero-superior portion of the pinna were made using methods previously described (9, 10, 11). The formula given by Isbell (9) for measuring the surface area of the finger tip from its volume was found applicable to the toe tip with a maximum error of 3 per cent. The finger tip as used in these studies is defined as that portion of the finger distal to a plane passing through the distal major dorsal and palmar skin creases. The toe tip is that portion of the toe distal to a plane passing through the distal major dorsal and plantar skin creases. The postero-superior portion of the pinna lies above a plane passing at right angles to the lateral surface of the pinna and slightly postero-superior to the portion of the pinna joining the scalp (see fig. 1).

RESULTS. Measurements of the rate of water eliminated from small areas of skin were made of 15 normal white adults (6 males and 9 females)

varying in age from 22 to 52 years. The subjects were studied at various times of the day while resting in bed and covered to satisfy each individual's comfort. The atmosphere of the room was controlled to maintain a temperature of 75°F. ±1 and a relative humidity of 50 per cent ±3. The parts for study were adjusted to the level of the heart. The subjects rested for an hour before collections were started. In 7 of the subjects the measurements were repeated after an interval of several days to weeks.

The mean amount of water collected from the right index finger tip of the resting subject was 1.86 mgm. per square centimeter per 15 minutes, the variations ranging from 3.82 to 0.81. The mean value for the right second toe tip was 1.18 mgm. per square centimeter per 15 minutes, the variations ranging from 2.16 to 0.52. The values for the postero-superior portion of the pinna of the right ear were found to possess a mean of 0.48
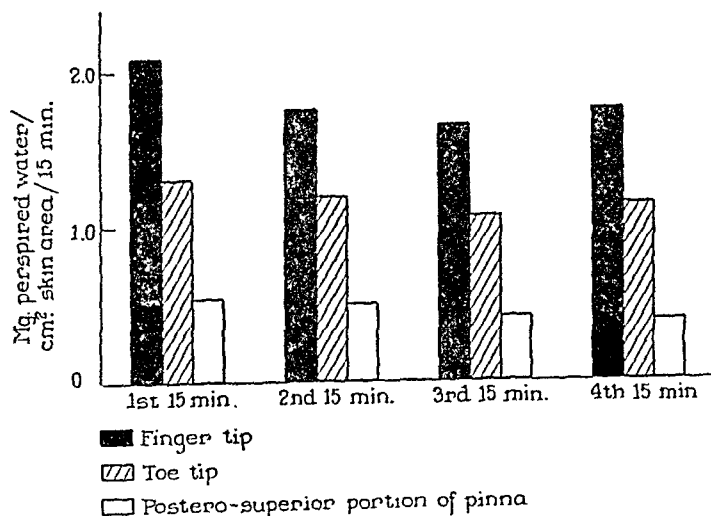
Fig. 3. Proportional rates of water perspired by finger tip, toe tip and pinna during four successive 15-minute periods.

mgm. per square centimeter per 15 minutes, the variations ranging from 0.69 to 0.29. The rate of the loss of water was greater from the finger tip than from the other two parts studied. The ratio of the mean rates of the elimination of water of the finger tips to toe tips was approximately three to two; of finger tips to postero-superior portion of the pinnae, approximately four to one. In two subjects the finger tip to toe tip ratio deviated markedly, being almost one to one. In most of the measurements it was found that the rate of the loss of water in the first 15 minutes was slightly greater than in the succeeding fifteen minute periods when there was a tendency to reach a constant level (fig. 3).

DISCUSSION. The method employed in these observations is open to certain objections; 1, because the atmosphere surrounding the parts studied was different from that surrounding the rest of the body; 2, because the

relative humidity of the room being 50 per cent, while that in the brass chambers was much less, may have tended to increase the rate of evaporation from the surface of the skin. The degree to which the transpiration of water and the activity of sweat glands were influenced by these factors is unknown. The nature of the gas surrounding the parts may have influenced the results but the degree was probably insignificant. In spite of these criticisms the method offers certain advantages. It is simple, accurate, and does not disturb the subject after the parts have been enclosed in the brass chambers. Controlling the flow of oxygen, turning the stopcocks, changing the collecting coils, weighing and drying the coils, and per-

TABLE 2

Rate of sweating (mgm./sq.cm. surface area/15 min.) in the right index finger tip of 13 normal white resting adults

SUBJECT NUMBER	AGE	SEX	SURFACE AREA FINGER TIP	FIRST 15 MINUTES	SECOND 15 MINUTES	THIRD 15 MINUTES	FOURTH 15 MINUTES	MEAN FOR 15 MINUTES
	years		*sq. cm.*					
1	30	M	11.64	2.56	2.27	1.72	1.63	2.05
2	40	M	10.64	2.27	1.84	2.07	1.97	2.04
3	33	M	11.47	1.83	1.31	1.31	1.74	1.55
4	27	M	12.06	2.67	1.92	1.44	1.41	1.86
5	28	M	12.94	2.26	3.82	2.13	1.74	2.49
6	39	F	9.81	2.75	2.08	1.78	2.01	2.16
7	30	F	8.77	1.38	1.37	1.40	1.74	1.47
8	40	F	9.04	1.64	1.97	2.01	2.03	1.91
9	23	F	10.25	1.95	1.83	1.88	1.87	1.88
10	52	F	10.17	1.01	0.93	0.81	1.12	0.97
11	25	F	10.51	2.81	2.03	1.51		2.12
12	30	F	8.54	1.79	1.55	1.43	1.43	1.55
13	50	F	10.58	2.16	1.85	2.15	2.40	2.14
Mean				2.08	1.75	1.66	1.76	1.86
Max.				2.81	3.82	2.13	2.40	2.49
Min.				1.01	0.93	0.81	1.12	0.97

forming the other necessary manipulations can be, and were, done far removed from the subject's bed. By modifying the size and shape of the various parts of the system the method can be used to study the rate of elimination of water from many normal or diseased surfaces.

The differences in the rate of loss of water from the parts studied were marked. The rate was two-thirds as rapid in the toe tip as in the finger tip and one-quarter as rapid in the postero-superior portion of the pinna as in the finger tip. The reason for these differences may be physiologic or anatomic or both. Since there are no satisfactory anatomical data (5, 12) on the number of sweat glands in the areas studied it is impossible to decide what the reasons are which account for the difference.

There are marked variations in the rate of water loss in different individuals (tables 2, 3 and 4). The rate of elimination of water from the

TABLE 3

Rate of sweating (mgm./sq.cm. surface area/15 min.) in the right second toe tip of 14 normal white resting adults

SUBJECT NUMBER	AGE	SEX	SURFACE AREA TOE TIP	FIRST 15 MINUTES	SECOND 15 MINUTES	THIRD 15 MINUTES	FOURTH 15 MINUTES	MEAN FOR 15 MINUTES	RATE OF SWEATING AS PER CENT OF FINGER TIP RATE
	years		*sq. cm.*						
1	30	M	10.99	1.09	0.79	0.69	0.70	0.82	39
2	40	M	10.70	1.48	1.40	0.97	1.06	1.23	58
3	33	M	10.51	1.41	1.41	1.17	1.35	1.34	90
4	27	M	9.97	1.15	1.18	0.82	0.91	1.02	61
5	28	M	12.12	1.56	2.16	1.58	1.47	1.69	68
6	39	F	10.92	1.62	1.09	0.89	1.09	1.17	52
7	30	F	8.81	0.81	0.81	0.52	0.96	0.78	51
8	40	F	10.50	1.00	0.84	0.89		0.91	43
9	33	F	11.23	1.02	1.21	0.90	0.94	1.02	55
10	52	F	8.67	0.82	0.89	0.82	0.91	0.86	92
12	30	F	8.33	1.13	0.90	1.12	1.07	1.06	70
13	50	F	9.17	1.35	1.30	1.29	1.49	1.33	64
14	22	F	8.55	2.08	1.52	1.73	1.72	1.76	
15	30	M	10.79	1.82	1.13	1.65	1.30	1.48	
Mean				1.31	1.19	1.07	1.15	1.18	
Max..				2.08	2.16	1.73	1.72	1.76	
Min.				0.81	0.79	0.52	0.70	0.78	

TABLE 4

Rate of sweating (mgm./sq.cm. surface area/15 min.) in the postero-superior portion of the pinna of 5 normal white resting adults

SUBJECT NUMBER	AGE	SEX	SURFACE AREA OF PORTION OF PINNA	FIRST 15 MINUTES	SECOND 15 MINUTES	THIRD 15 MINUTES	FOURTH 15 MINUTES	MEAN	RATE AS PER CENT OF FINGER TIP RATE
	years		*sq. cm.*						
1	30	M	13.21	0.64	0.60	0.57	0.50	0.58	30
2	40	M	13.86	0.66	0.69	0.42	0.40	0.54	26
3	33	M	13.02	0.50	0.44	0.41	0.42	0.44	29
4	27	M	13.28	0.41	0.38	0.41	0.30	0.38	23
13	50	F	13.60	0.49	0.41	0.29	0.34	0.39	16
Mean				0.54	0.50	0.42	0.39	0.48	
Max.				0.66	0.69	0.57	0.50	0.58	
Min.				0.41	0.38	0.29	0.30	0.38	

finger tip of subject number 2, for instance, was almost twice as rapid as from subject number 10. Although the rate of the elimination of water

varied within the same individual from time to time the level remained fairly constant. The rates are apparently correlated with the emotional type of the subject. Phlegmatic subjects lost water less rapidly than excitable ones.

SUMMARY

A method is described for measuring the rate of water loss from small surfaces. The method consists in passing dry oxygen through chambers covering the surfaces and then conducting the moisture-containing oxygen through cold aluminum coils. From the difference in weight of the coils before and after the passage of the oxygen, the amount of water lost is learned. The method is accurate to 2.6 per cent. This error can, however, reach 9 per cent when less than 6 mgm. of water are measured, but such low values were not encountered.

The rate of the elimination of water was studied from the right index finger tip, right second toe tip and postero-superior portion of the right pinna of 15 white, normal, resting adult subjects. The mean rate of water loss was found to be 1.86 mgm. per square centimeter per 15 minutes for the finger tips, 1.18 mgm. for the toe tips and 0.48 mgm. for the pinnae. The rate of water loss in the toe tips was approximately two-thirds as rapid as in the finger tips and the rate for the pinnae was only one-quarter as rapid as that for the finger tips.

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THE LACTIC ACID MECHANISM AND CERTAIN PROPERTIES OF THE BLOOD IN RELATION TO TRAINING

S. ROBINSON AND P. M. HARMON

With the technical assistance of E. S. TURRELL and F. O. MACKEL

From the Department of Physiology, Indiana University, Bloomington

In work where the rate of energy expenditure exceeds the capacity for oxidative processes, a considerable portion of the energy supplied anaerobically depends on the "lactic acid mechanism" for contracting an O_2 debt, an expression applied by Margaria, Edwards and Dill (1933). In strenuous athletics the lactic acid mechanism as a source of anaerobic energy in muscular contraction is of major importance, particularly in maximal effort of short duration. The observations of Herxheimer (1924) that training increases the alveolar CO_2 tension, and of Wissing (1926), Rehberg and Wisseman (1927), Ewig (1928) and Full and Herxheimer (1926) that training increases the alkaline reserve indicate that training may increase a man's tolerance for lactic acid in severe anaerobic work. Dennig (1937) has found that work capacity is increased when the alkaline reserve is raised by ingesting mixtures of sodium citrate, sodium bicarbonate and potassium citrate. Robinson, Edwards and Dill (1937) found the basal alkaline reserve in 5 champion mile runners to be the same as in non-athletes. Thörner (1932) found that training had no effect upon the alkaline reserve of dogs. Recent unpublished observations by the present authors made on 15 highly trained college runners under basal conditions show an average alkaline reserve of 21.5 mM. per liter of blood at 40 mm. Hg pCO_2. This average is no higher than the generally accepted averages for non-athletes and every individual athlete was within the normal range. In basal alveolar air samples from these college athletes and the 5 champion runners we found a mean CO_2 tension of 41.9 mm. Hg with the highest value 45.

The present study is designed to determine the effects of training upon the lactic acid mechanism ·in work and its relation to certain properties and constituents of the blood; the alkaline reserve has received particular attention because of the above mentioned variance in results. As subjects 9 non-athletic college students, ages 18 to 22, were chosen from 40 applicants who went through preliminary tests on the treadmill. The men chosen possessed varied athletic ability from the poorest to the best of the

40 applicants.   To insure coöperation they were paid for their time.   The training was continued for a period of 6 months and consisted of a carefully supervised running program with four workouts on the track each week during the first month and thereafter workouts on Tuesday, Wednesday and Thursday of each week with a time trial every Saturday to measure progress.   The Saturday trials were at $\frac{1}{2}$, $\frac{3}{4}$, 1 and 1$\frac{1}{2}$ miles in rotation. About once a month a mid-week trial in a sprint was held to test speed. The training program each week was planned to give the men the best preparation for the coming trial, with over-distance running one day and pace work and speed work on the other two days.   The men performed as much running during training as their legs could stand without developing handicapping soreness.   Gymnasium work was included each day for general conditioning.   An example of the progress made in training was the consistent lowering of the men's time for the mile run: in December the 9 men averaged 6 minutes and 24 seconds on the mile as compared with 5 minutes and 15 seconds the following April.

Observations in the laboratory were made on the men before training started and at regular intervals during the training period.   The work tests in the laboratory were performed on a motor driven treadmill and consisted of: 1, a standard 15-minute walk at 5.6 km. per hour on an 8.6 per cent grade with finger blood being drawn for lactate and sugar after 10 minutes of walking when the subjects had attained a steady state; 2, a 10-minute run on the level at a moderate pace which was 12.9 km. an hour for 7 of the men and 14 km. for the two best runners; blood for analysis was drawn from an arm vein 5 minutes after the end of the run; 3, a run severe enough to exhaust the men in 3 to 5 minutes.   During the training period each time a man became able to complete 5 minutes of the exhausting run the grade or speed or both were increased for him in the next test in an attempt to keep the work just severe enough to exhaust him in 4 to 5 minutes, his goal being to complete the 5 minutes.   As in the 10-minute run blood was drawn 5 minutes after the exhausting run.   (We have used the concentration of lactic acid in venous blood drawn 5 minutes after stopping these runs to indicate the extent to which the lactic acid mechanism has been brought into action: according to Margaria, Edwards and Dill (1933) the lactate at that time has become uniformly distributed between tissues and blood.   Evidence of Hill (1928), Evans (1930) and Newman (1938) support this assumption.)   All repetitions of the 15 minute walk and the 10-minute or moderate run for each man were at the same intensity as in the original tests.   About once a month each man repeated a 5 minute run on the treadmill at the same speed and grade as the run which was originally exhausting for him.   Basal alveolar air and venous blood samples were drawn with the men in the recumbent position at the end of basal metabolism determinations.

Blood lactate was determined by the method of Edwards (1938), blood sugar by the method of Folin and Malmros (1928) modified for the photo-electric colorimeter, and plasma protein by micro-Kjeldahl analysis. HbO_2 capacity and alkaline reserve were determined by equilibration of blood with O_2 and CO_2 pressures of 200 and 40 mm. Hg respectively at 37°C. as described by Dill in Henderson's book (1928).   The blood gases were
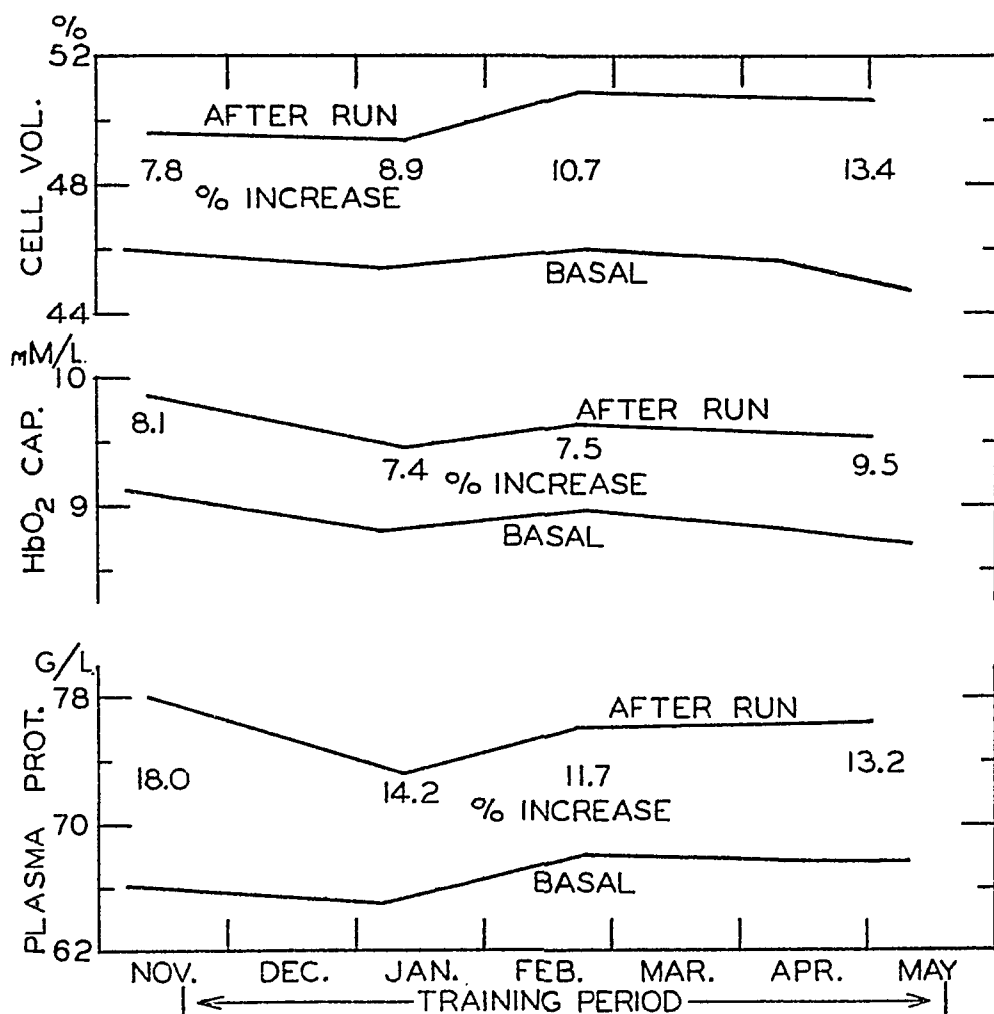
Fig. 1. The effects of training on the blood of 9 men.   Mean values in the basal state and 5 minutes after exhausting work.

analyzed in the Van Slyke apparatus.   The alkaline reserve as used in this paper is the CO_2 content of oxygenated whole blood at 40 mm. Hg CO_2 tension with temperature 37°C.   Alveolar air samples were collected in the basal state as described by Bock and Field (1924) and during work by the method of Henderson and Haggard (1925), analysis being made with the Haldane apparatus.

The basal state.   Figure 1 reveals that training had no significant effect

upon the HbO_2 capacity or cell volume of the blood nor upon the plasma protein in the basal state. The mean values of these constituents are all very close to generally accepted standards. Certainly there is no tendency for any of them to increase with training. Variations of the means of the 3 components tend to coincide with each other. Variability of individual values is about the same in the successive determinations. For example, the extreme basal HbO_2 capacities in November were 7.9 and 9.6 mM. per
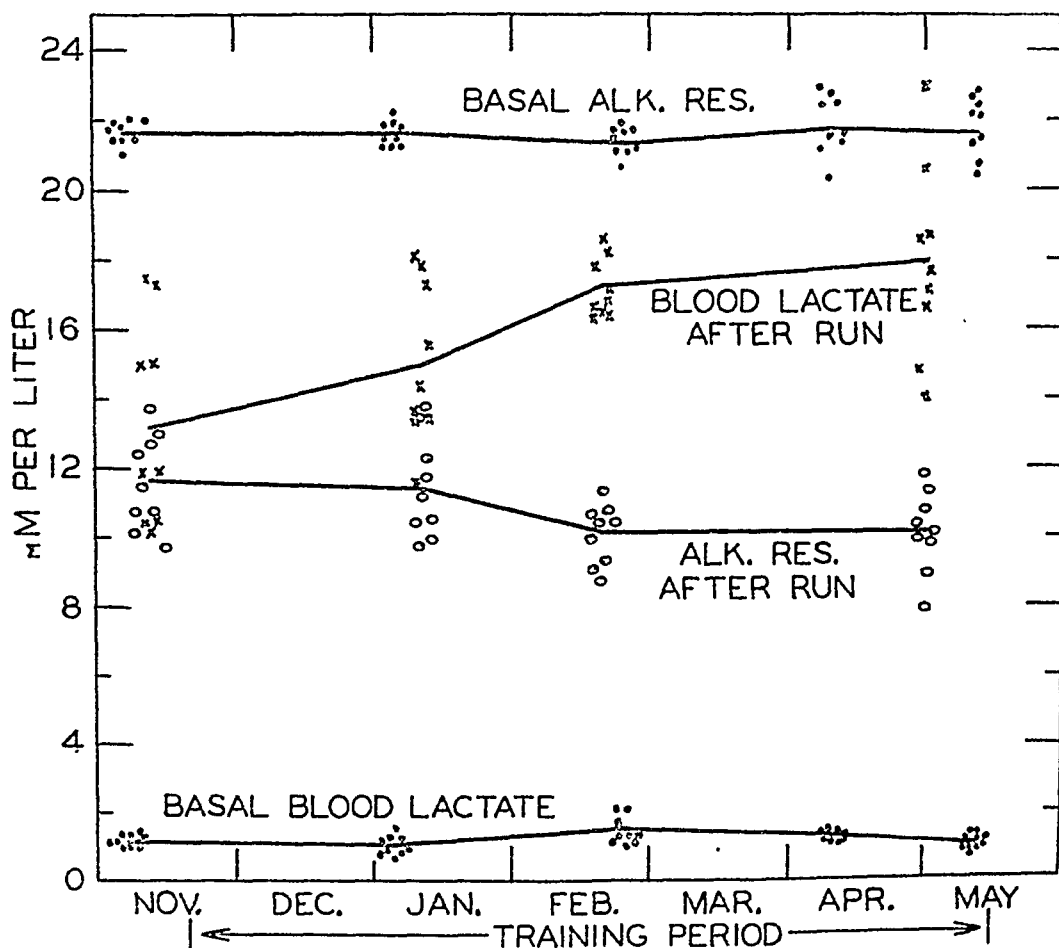
Fig. 2. The effects of training on the blood of 9 men. Individual and average values of alkaline reserve and lactic acid in the basal state and 5 minutes after exhausting work.

liter as compared with 7.9 and 9.5 by the same individuals in May. Figure 2 includes scatter diagrams of basal blood lactate and alkaline reserve. Lactate shows no change of the average or of the variability resulting from the training. On the average the alkaline reserve remained unchanged but it showed a greater variability in the later months of training. The values are all well within the usual limits for untrained people according to the data of Hurxthal, Bock, Talbott and Dill (1929), Dill,

Edwards and Consolazio (1937), Robinson (1938) and of Dill, Wilson, Hall and Robinson (1940).   Our data thus fail to show an increase in alkaline reserve in training as was reported by the European workers cited above. Dietary effects on the alkaline reserve should be considered as a possible explanation of the differences between the results of the European and American observers.   The training diet of American athletes usually contains about the same proportion of alkali forming foods as their non-training diet.   The only dietary change which was made by our subjects during the entire experiments was that the men took 60 grams of gelatin a day for a period of 6 weeks during the middle of the training period.   This caused no change in the properties of the blood.   Figure 5 includes the mean values of CO_2 tension in basal alveolar air.   There was an average decline of about 1.5 mm. Hg after training started which was in the opposite direction from the change noted by Herxheimer (1924) and by Ewig (1928). Since Bock and Field (1924), Bock et al. (1929) and Robinson (1938) have proved that resting alveolar air gives a close approximation of the arterial CO_2 tension and there was no change in the CO_2 combining capacity of the blood we may assume that the acidity of arterial blood did not increase in these men and therefore there was no increase either in CO_2 tension or acidity as a stimulus for resting respiration.

Exhausting work.   Figure 1 shows the percentage changes of HbO_2 capacity and cell volume of blood and of protein in plasma in the adaptation from rest to exhausting work.   In the initial test before training started the percentage increases in cell volume and in hemoglobin are the same.   Increments of hemoglobin resulting from the work show no significant changes which are related to the training period.   Increments in cell volume resulting from the work change gradually from 7.8 per cent in the initial test to 13.4 per cent in the final test.   This discrepancy between changes in hemoglobin and in cell volume is accounted for by the fact that the men accumulated more lactic acid in work as the training period advanced (fig. 2).   Dill, Edwards and Consolazio (1937) have shown that as the pH of blood decreases the cell volume increases.   In the initial test the plasma protein increased by 18 per cent as the result of work.   If we assume that as the plasma lost water to the tissues in this experiment no protein passed from the circulation, the actual increase in hemoglobin corresponded to the increment calculated from the concentrations of plasma protein and cells.   This relationship confirms the observations of Dill, Talbott and Edwards (1930) on non-athletic subjects performing sub-maximal work.   In subsequent tests during training the relationship was not so exact in our subjects, water loss from the plasma not being great enough to account entirely for the changes in hemoglobin.

Figure 2 shows, in 4 experiments, the effect of the exhausting run upon the blood lactic acid and alkaline reserve.   It will be noted that the mean

values of blood lactate after the run increase progressively from 13 mEq. per liter before training to 17.9 at the end of the training period and that the alkaline reserve is correspondingly decreased in each set of experiments. Individual values of lactate and alkaline reserve, plotted in figure 2, show much wider variation than the resting values. Though there was a general increase in ability to accumulate lactate in these experiments the individuals were not capable of repeating their highest previous values every time they tried. The fact that the men became capable of utilizing the lactic acid mechanism more completely during the training period is one of the most interesting changes observed in this study. Since there was no increase in the amount of available base for buffering the acid, as indicated
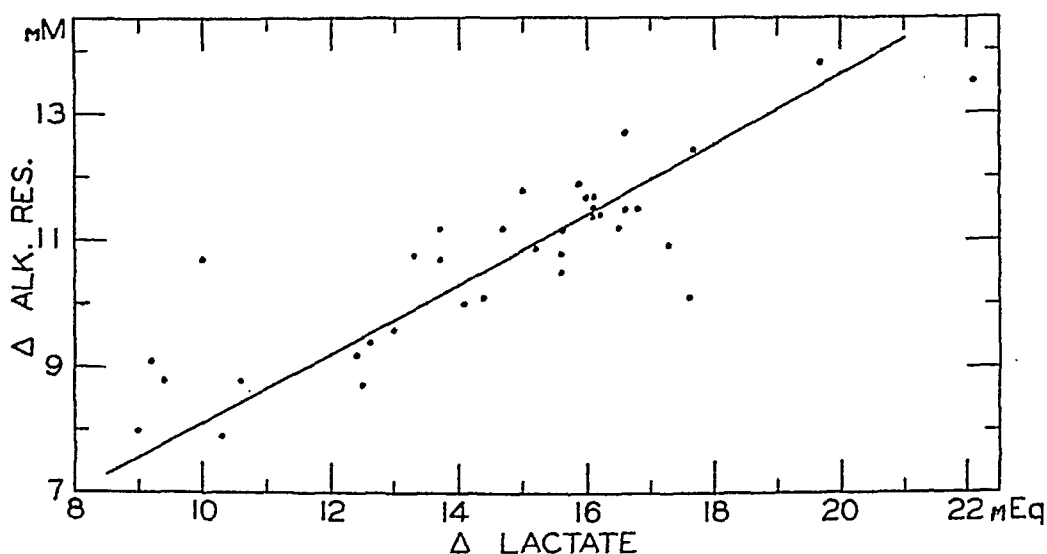
Fig. 3. Adaptation to the exhausting run. Blood lactic acid increases above the basal values (Δ-lactate) are plotted against the corresponding decreases in alkaline reserve (Δ-alkaline reserve) for the 36 individual experiments. The straight line calculated by the method of least squares indicates the relationship.

by basal alkaline reserve, the higher lactates accumulated after the runs during training made the reserve alkali lower than in the initial tests. . The decrease below the basal level of alkaline reserve in the run was not equivalent to the rise in lactate, the latter being greater by about 2 mEq. per liter before training and by 5 mEq. after 6 months of training. As the lactic acid concentrations became higher in the later experiments more of it was buffered by base from proteinate and the pH necessarily became lower.

It is interesting to make a further analysis of the relation of blood lactate to alkaline reserve in work. Figure 3 shows, for the exhausting run, the individual blood lactic acid increases above the basal values (Δ-lactate) plotted against corresponding decreases in alkaline reserve (Δ-alkaline

reserve). In all but one of the 36 individual determinations the change in lactate was greater than the change in alkaline reserve. A straight line calculated by the method of least squares indicates that in this range of concentrations a change in Δ-lactate from 10 to 20 mEq. per liter corresponds to a change in Δ-alkaline reserve from 13.6 to 8.1 mM. Reports in the literature are controversial on this relationship. Mellanby and Thomas (1920) and Evans (1922) found that changes in alkaline reserve were smaller than corresponding additions of lactic acid to drawn blood. In Evans' data the difference was greater at physiologically high concentrations of lactate than at low concentrations. His results in the physiologically high range were very close to ours, a change in Δ-lactate of 10 mEq. corresponding to a change of 5 mM. in Δ-alkaline reserve. Barr, Himwich and Green (1923) found wide variations in 6 exercise experiments on men and that Δ-lactate was higher in only 2 cases. Dill et al. (1930) found that Δ-alkaline reserve was generally greater than Δ-lactate in exercising men where the blood lactate increased up to 6 mEq. per liter. Dennig et al. (1931) found the changes to be about equal in men at work where the blood lactate increased to about 10 mEq. The conditions of these authors were different from ours in two respects: their lactate values were lower, and in the last two papers the analyses were made by the method of Friedemann, Cotonio and Shaffer (1927) which tends to give lower recoveries of blood lactate than the method as revised by Edwards (1938) which was used in this study.

Figure 4 includes the mean blood lactate determinations after 9 sets of exhausting runs, including the 4 tests in which alkaline reserve was also measured. This curve shows the same increase of lactate with training which was described above. There is one break in the curve at the end of January which is probably related to the fact that the tests were made during examination week when the men were tired and nervous and for that reason were either unwilling to extend themselves or incapable of doing so as completely as in the preceding test. The mean blood lactate of 13 mEq. per liter in the initial test is very close to averages reported by Robinson et al. (1941) of initial tests on 83 non-athletic young men in similar exhausting runs on the treadmill.

The increased ability of the men to accumulate lactate in exhausting work was accompanied by a decline in the average alveolar CO_2 tension during work, an increase in the excess (above basal) O_2 consumed in the first 15 minutes of recovery, and no change in blood sugar concentrations (fig. 4). The lower alveolar CO_2 values in the later experiments were due to overventilation of the lungs associated with the greater concentrations of lactate. This accounts in part for the increase with training in the tolerance of the men for lactate. Probably improved circulation to the leg muscles also played a part by removing and distributing lactate faster so

that the organism as a whole could buffer more of it. There is also a possibility that the men underwent changes in sensitivity to acidosis and in determination and confidence. The greater rate of O_2 consumption in early recovery was probably associated with increased circulation, due in
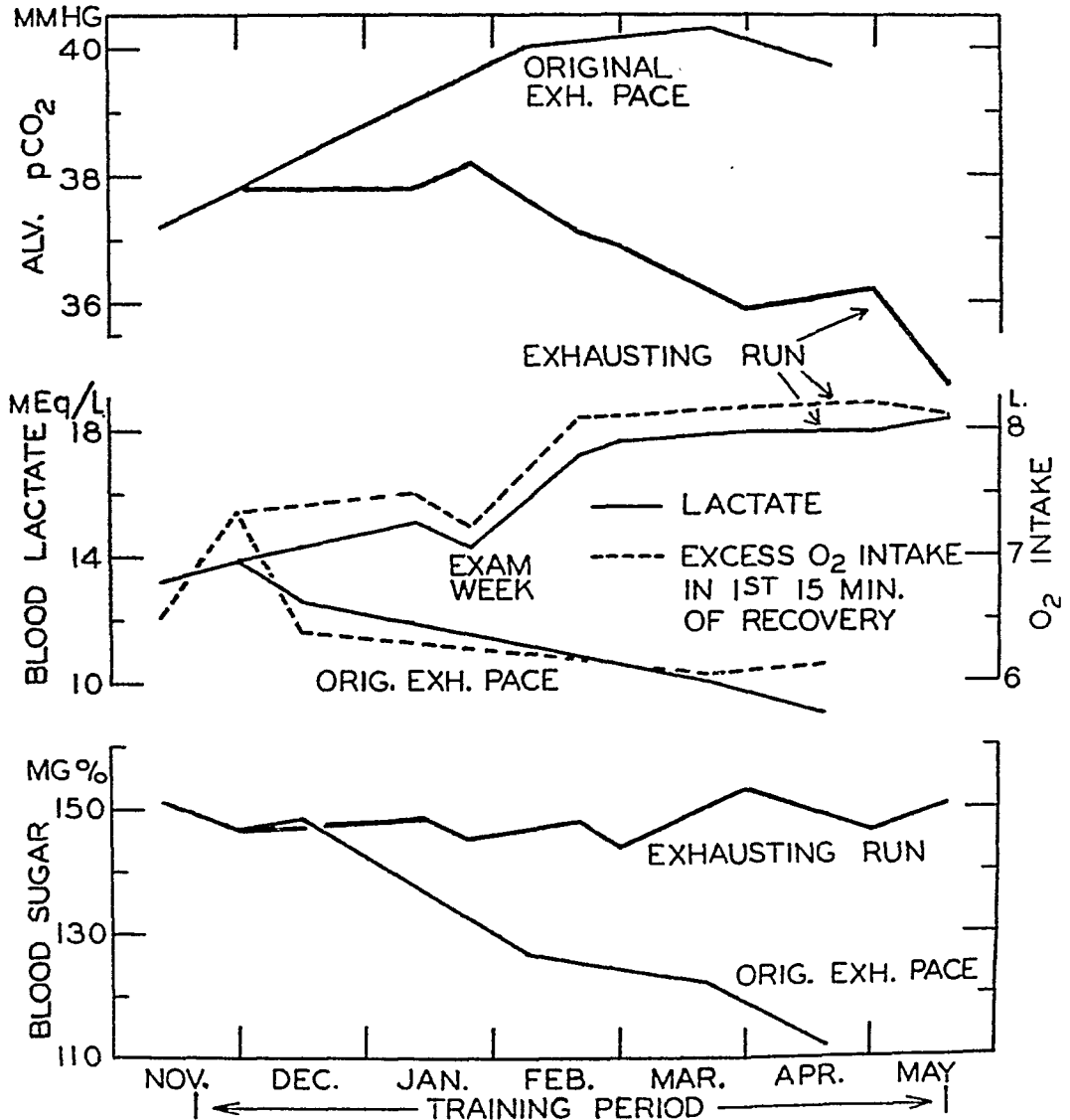
Fig. 4. The effects of training on the mean values of blood sugar and lactic acid 5 minutes after exhausting work, of alveolar CO_2 tension during the same work, and of excess O_2 intake (above basal) during the first 15 minutes of recovery. The same data are given for repetitions, during the training period, of the run at the same pace which was originally exhausting for the 9 men.

part to the influence of the high concentration of lactate in the body and in part to improvement of the circulatory mechanism with training. The fact that the blood sugar values did not increase along with lactate may indicate that the later runs were no more of a shock or stimulus to the

"emergency mechanism" than were the earlier runs when the accumulated lactates were lower but the men were not accustomed to them.

Not all of the subjects were able to complete 5 minutes of running at the speed and grade assigned in the initial test. The poorest runner required two months of training to accomplish this. At intervals this same 5-minute run was repeated even after it became submaximal and the men had all progressed to harder speeds and grades for their exhausting work. Figure 4 shows that after the 2nd test there was a rapid decline in blood lactate following this run from a mean of 13.7 mEq. per liter in December to 9 mEq. in April. This decline in lactate means that the men were able to complete the run with a decreasing percentage of the energy being supplied by anaerobic processes. It implies an increase in the maximal ability of the men to consume O_2 which is related both to improvement of circulation and of oxidative processes in the muscles, and perhaps an increase in skill which would decrease the total amount of energy required to maintain the pace. (Our data on O_2 consumption and efficiency will be presented in another paper.) Corresponding to the decline in lactate in repeated tests at this original pace there was a decline in the excess (above basal) O_2 consumed in the first 15 minutes of recovery though the curves do not follow parallel courses. The alveolar CO_2 tension increased from an average of 37.2 to 40.3 mm. Hg as the lactates become lower. This is in accord with the results of Schneider and Ring (1929) who have found that training increases the intensity of work which a man can perform before the concentration of CO_2 in expired air begins to drop. The blood sugar declined from 150 to 112 mgm. per cent indicating that the test was not eliciting the "emergency mechanism" so extensively in the later experiments.

Submaximal work. Figure 5 shows the effects of training upon the response to the standard walk and the 10-minute or moderate run. In the walk blood lactate and blood sugar were only moderately elevated and showed downward trends with training—blood sugar did not rise above the basal level in the late experiments. It should be remembered that the men were being trained for running and not for walking. We have found that distance runners after several years of training perform this walk with no elevation of blood sugar or lactate above basal levels. A low blood lactate in this work indicates a superior oxygen supply to the tissues. Alveolar CO_2 tension in the walk made no consistent change, remaining about the same as the resting values before and during training. The fact that alveolar CO_2 in the walk was the same as at rest is associated with the fact that no marked acidosis was developed—after 8 minutes of walking when the men had attained a steady state all energy for the work was supplied aerobically. The samples of alveolar air were collected near the end of the 15 minutes of walking. The absence of a training effect on

alveolar CO_2 in this work lends support to the assumption that the sensitivity of the respiratory mechanism to CO_2 is not affected by training.

In the initial moderate run blood sugar and lactic acid were both significantly elevated. Blood sugar declined rapidly with training and was only slightly above the basal value in the last 3 experiments. The decline of lactate was more gradual and at the end there was room for continued
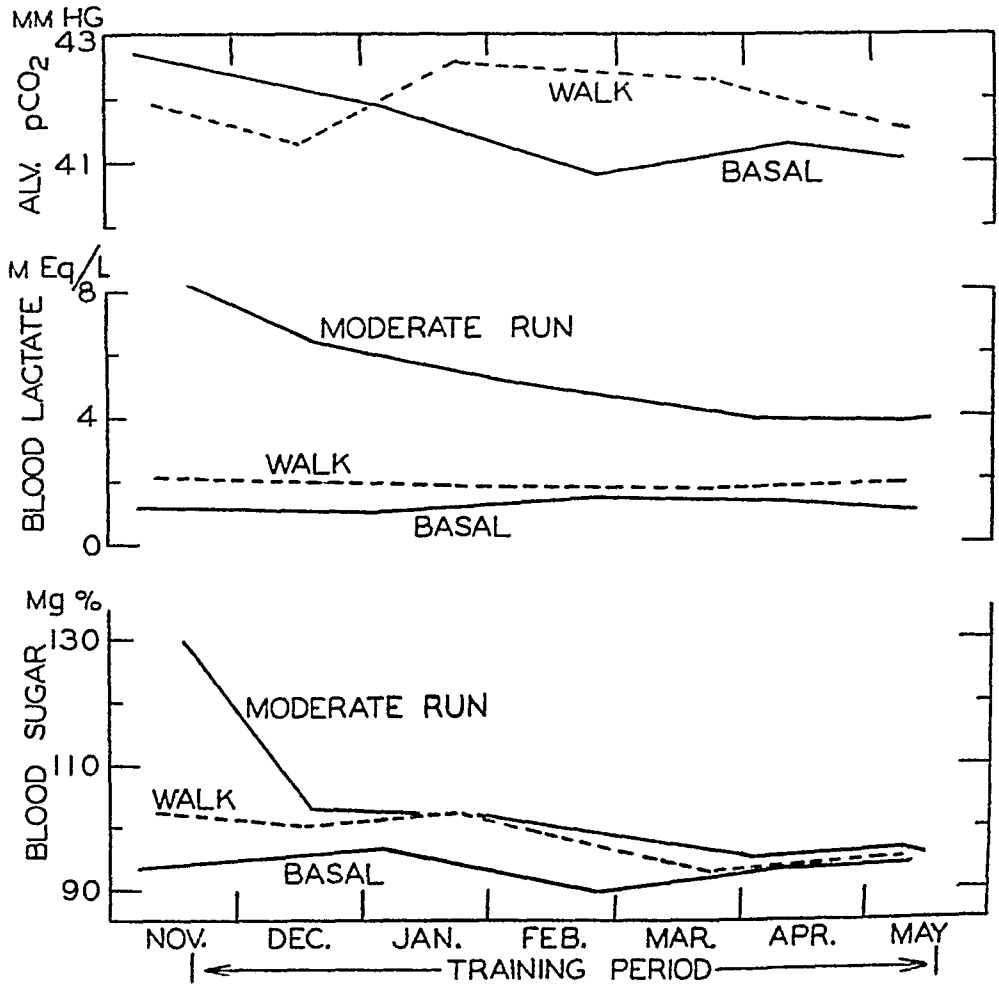
Fig. 5. The effects of training on the mean values of blood sugar, lactic acid and alveolar CO_2 tension in submaximal work.

improvement with further training. A decline of blood lactate with training in a less severe run than this has been previously noted by Edwards, Brouha and Johnson (1940). We have found that distance runners after several years of training perform this run with no elevation of blood sugar or lactic acid above resting levels. In this run, as in the walk, a low blood lactate indicates a superiority in O_2 supply to tissues. In the initial test the average O_2 requirement for maintaining the run was 90 per cent of the

average maximal capacity of the men for O_2 consumption as compared with 73 per cent at the end of the training period. A low blood sugar indicates that the "emergency mechanism" has not been elicited as it is when the organism is subjected to severe stress.
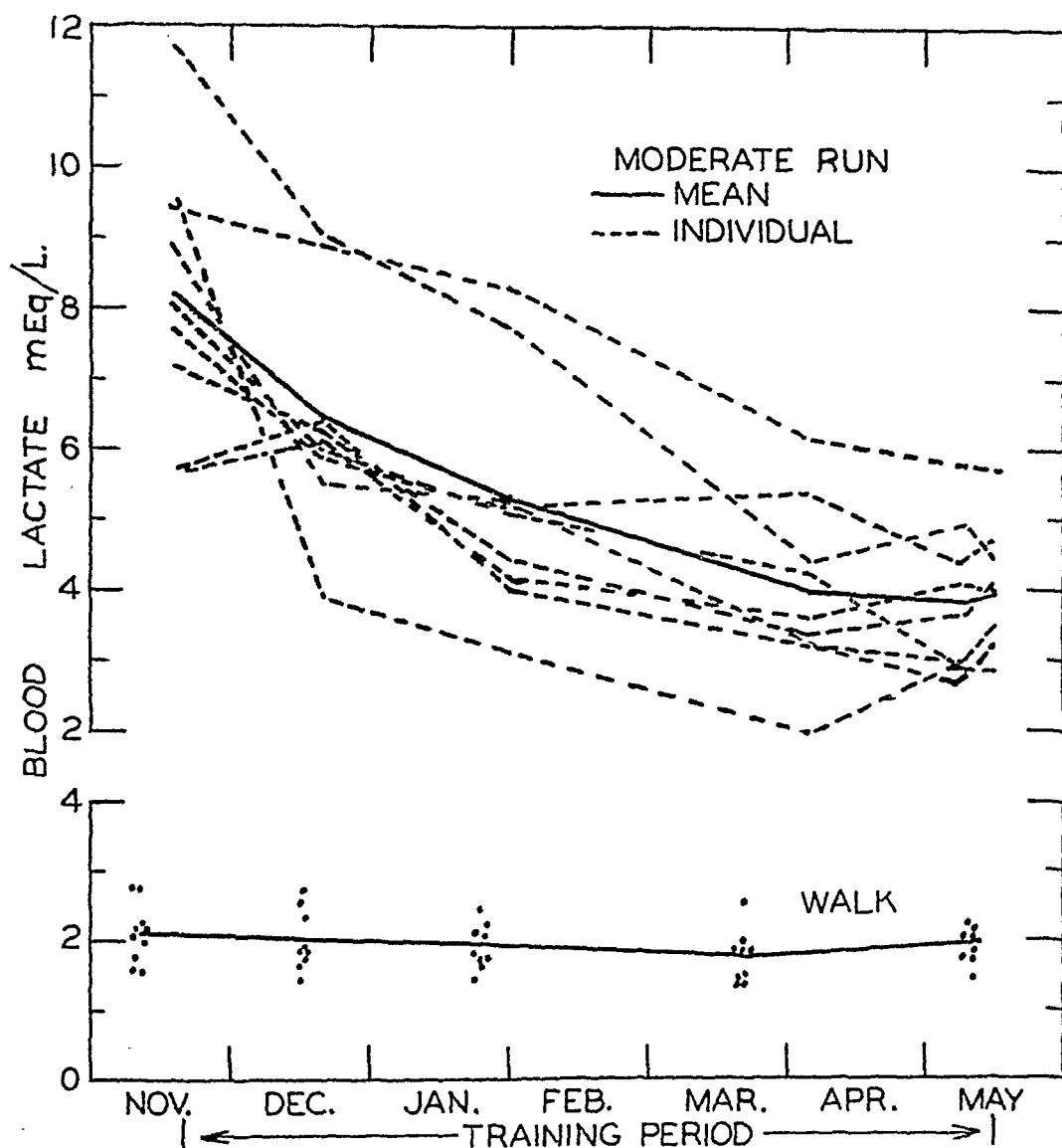
Fig. 6. The effects of training on the individual and mean records of blood lactic acid in two grades of submaximal work.

Individual variations of blood lactate in the walk and moderate run are shown in figure 6. The significant characteristic of the walk is the narrow range of variation. In the moderate run it is interesting to note the consistency with which the individuals improved. In the first 5 months there were only three individual tests in which a subject failed to do as well as

in his preceding test.   This characteristic of the individual curves is due partly to the fact that the intervals between successive tests were long enough to allow significant training effects.

SUMMARY

1. Strenuous athletic training for 6 months did not affect the basal HbO_2 capacity, plasma protein, blood lactic acid, blood sugar, alkaline reserve or alveolar CO_2 tension in 9 men.

2. The ability of the men to accumulate lactic acid during anaerobic work increased with training; during the same work there were corresponding declines in alkaline reserve and alveolar CO_2 tension.   The changes in blood sugar and HbO_2 capacity caused by the work remained about the same throughout training.

3. During grade walking blood sugar and lactic acid declined slightly with training.   Alveolar CO_2 tension remained unchanged and at about the same level as the basal values.

, 4. In submaximal running the blood lactate and sugar declined significantly with training.

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CHEMICAL CHANGES IN THE BRAIN PRODUCED BY INJURY AND BY ANOXIA[1]

WILLIAM E. STONE,[2] CLYDE MARSHALL AND LESLIE F. NIMS

From the Laboratories of Neurophysiology, Neuro-Anatomy, and Physiology, Yale University School of Medicine

The effects of cerebral injury and of cerebral anoxia on the cortical pH and electrical activity and on the concentrations of lactic acid, inorganic phosphate, phosphocreatine, pyrophosphate and "hexose phosphates" of the cerebral cortex have been investigated.

PROCEDURES. The cerebral hemishperes of cats anesthetized with nembutal or dial were exposed and electrodes suitable for the measurement of electrical activity and pH (1) were placed on the cortex. The electrical activity and pH were continuously recorded and when the character of either record indicated that significant alteration of the chemical pattern of the brain might be expected the area under observation was frozen *in situ* with either precooled metal blocks or directly with liquid air. The frozen tissue was then removed and kept in liquid air until chemical analysis was begun.

One gram of tissue consisting principally of grey matter was taken for analysis. Lactic acid and the phosphate fractions were determined by methods already described (2). The pyrophosphate fraction is believed to be almost entirely adenylpyrophosphate, while that labelled "hexose phosphates" includes hexose-6-monophosphate, hexose diphosphate and triose phosphates, if present, and possibly other compounds as well.

RESULTS. 1. *Effects of injury.* A small portion of the cerebral cortex (5 x 15 mm.) was frozen with a precooled metal block and removed. Somewhat later a sample was taken in the same way from an adjacent area. On examining the two samples chemically it was found that the second sample had a greatly increased lactic acid concentration. The results of several such experiments are summarized in table 1.

In a second group of experiments a portion of the cerebral cortex was frozen with liquid air and a block of the cerebral tissue was removed. At

a later time a homologous area of the contralateral hemisphere was sampled in the same way. It was found that the second sample contained greater amounts of lactic acid and of inorganic phosphate than did the first. The phosphocreatine content and the pH of the second region were both diminished. The acid shift in the contralateral hemisphere following the injury to the first hemisphere could be prevented if the respiration of the animal was increased at the time the injury was made, but essentially the

TABLE 1

Effect of freezing and extirpation of one area on the lactic acid content of an adjacent area

AREA	LACTIC ACID OF THE CORTEX				
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
	mgm./100 gm.	mgm./100 gm.	mgm./100 gm.	mgm./100 gm.	mgm./100 gm.
First.............	38	28	37	56	31
Second...........	62	74	91	86	101

TABLE 2

Effect of freezing and removal of part of one hemisphere on the contralateral hemisphere

WEIGHT OF CAT	HEMISPHERE	RESP. VOL. AT 28/MIN.	pH	LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
kgm.		cc.		mgm. per 100 gm.	mgm. P per 100 gm.	mgm. P per 100 gm.	mgm. P per 100 gm.	mgm. P per 100 gm.
3.23	First	40	7.24	7	8.1	14.9	13.2	23.9
	Second	40	6.96	29	9.9	12.2	14.4	23.1
2.97	First	60	7.33	27	8.6	11.9	10.7	24.5
	Second	60	6.90	82	12.3	9.9	12.0	22.1
3.24	First	75	7.10	72	11.9	9.0	11.7	17.7
	Second	75	6.70	109	13.3	7.8	9.5	17.0
3.27	First	50	7.33	22	15.2	13.1	11.8	19.1
	Second	100	7.16	86	12.7	9.4	10.9	18.6
2.87	First	60	7.02	40	12.3	11.7	12.1	19.8
	Second	100	7.08	95	17.1	7.3	9.9	13.9

same changes occurred in the concentrations of the lactic acid and phosphate fractions. The results are summarized in table 2.

In a few experiments the pH electrode alone was used as an indicator of chemical change. It was found that either freezing or cutting of cortical tissue could produce changes in the chemistry of adjacent and more remote regions. While the quantitative effect was variable, the effect produced in the area under observation seemed to be dependent on the distance from the injured region and the extent of the injury. It was also noticed that less disturbance was produced to the rest of the cortex if the cerebral tissue was removed by "suction" rather than by cutting.

2. *Effects of reduced cerebral blood flow.* A differential blood flow to the two cerebral hemispheres of a cat was achieved by the cutting of one common carotid artery. As the animal bled from the cut vessel both the electrical activity and the pH of the ipsilateral cortex decreased rapidly. Both hemispheres were frozen simultaneously a few minutes later, at a time when the contralateral cortex began to show a slight reduction in its electrical activity. The chemical results of two such experiments are summarized in table 3. It is clear that the hemisphere with the decreased blood supply had a greater concentration of lactic acid and of inorganic phosphate and a lesser concentration of phosphocreatine than did the control.

3. *Effects of low oxygen.* Three series of animals were used. The first series served as a control for the second and third and gave the concentrations of various chemical fractions that obtain in a nembutalized

TABLE 3

Effect of a reduction of blood flow on the chemical pattern of the cerebral cortex. Left common carotid artery cut

HEMISPHERE	pH		LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
	Before	After					
			mgm. per 100 gm.	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*
Right.........	7.21	7.20	30.9	11.7	11.5	13.4	14.8
Left..........	7.09	6.87	71.0	16.8	9.5	13.2	16.2
Right.........	7.03	6.97	30.9	13.4	9.0	12.8	15.8
Left..........	6.98	6.70	59.0	17.9	6.4	12.0	15.7

cat breathing air. The values obtained for the concentrations of lactic acid, inorganic phosphate and phosphocreatine agree with results presented by Avery, Kerr and Ghantus (3).

The second series included those animals which were allowed to breathe nitrogen (for 2 to 3 min.) until their cerebral cortices became and remained electrically silent for 20 to 30 seconds. At this time samples, obtained by freezing with liquid air, were removed for analysis.

Animals comprising the third series also breathed nitrogen. These, however, were artificially ventilated with air soon after the electrical activity of the brain had ceased, so that the period of electrical silence (20 to 30 sec.) was equal to that of the preceding series. The brains were frozen some two and a half minutes later at a time when the electrocortico-gram had nearly returned to its pre-anoxic appearance. The chemical results obtained in these three series of animals are summarized in table 4.

Among the chemical changes to be noted are: 1. An alkaline shift in

pH during the period of anoxia in spite of an increased concentration of lactic acid.   2. The reciprocal changes in inorganic phosphate and phosphocreatine.   3. The "rebound" in the levels of inorganic phosphate and phosphocreatine during the recovery period.

TABLE 4

Chemical pattern of the cerebral cortex before, during, and after a short period of breathing nitrogen

EXPERIMENT NUMBER	BRAIN pH			LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
	Before anoxia	During anoxia	After anoxia					
				mgm. per 100 gm.	mgm. P per 100 gm.	mgm. P per 100 gm.	mgm. P per 100 gm.	mgm. P per 100 gm.
43	7.37			23.3	10.5	11.3	19.9	27.1
55	7.30			16.8	11.4	13.1	12.4	17.0
41	7.23			13.5	11.5	13.5	21.1	22.2
39	7.19			8.9	8.4	13.4	15.4	24.6
54	7.18			20.2	11.1	14.6	11.0	10.5
36	7.18			16.3	12.2	14.6	15.6	21.1
45	7.00			16.0	11.6	12.4	26.5	21.1
40	6.98			20.3	11.9	11.3	22.4	25.2
Average.....	7.18			16.9	11.1	13.0	18.0	21.1
77	7.33	7.50		61.4	14.3	7.6	18.3	20.0
78	7.03	7.13		55.9	13.7	10.5	16.1	18.5
79				58.0	14.7	9.0	25.5	23.2
81	7.02	7.20		50.6	13.6	7.9	22.3	28.3
82	7.30	7.33		52.3	15.3	7.2	23.9	26.2
88	7.20	7.25		48.8	14.7	6.0	11.8	16.2
Average.....	7.18	7.28		54.5	14.4	8.0	19.7	22.1
83	7.29	7.48	7.36	16.9	7.1	14.7	25.4	24.9
84	7.28			31.8	7.6	15.6	21.9	30.9
85	7.24	7.32	7.40	31.6	6.6	15.3	27.6	29.0
87	7.16	7.21	7.28	17.0	6.7	15.4	9.5	18.5
89	7.32	7.35	7.29	28.6	7.1	11.0	10.7	14.9
Average.....	7.26	7.34	7.33	25.2	7.0	14.4	19.0	23.6

A "rebound" in the electrical activity following the anoxic period was also noted similar to that already described by Bremer and Thomas (4), and it may well be that these chemical and electrical phenomena are causally related.

DISCUSSION.   The fact that injury to one portion of the cerebral cortex can produce changes in the chemistry and presumably in the function of adjacent regions must be borne in mind in attempting to evaluate the

functional deficits resulting from a supposedly local lesion. Some of the chemical effects are undoubtedly due to unavoidable alteration in the cerebral blood flow, as the similarity of the results presented in tables 2 and 3 attests.

The pH shifts reported in the several experiments cannot be completely accounted for. The change in lactic acid is at times proportional to the observed pH change, as in the experiments reported in the first part of table 2, but lactic acid does not quantitatively account for the observed pH changes if the carbon dioxide tension is assumed to be maintained at a constant level. Simple calculations will show that the phosphate fractions contribute little to the acid-base shifts. Thus carbon dioxide is left as the most likely regulator of cortical acidity, though other as yet undetermined acid or alkaline substances may play a significant rôle in this regulation. The alkaline shift during nitrogen inhalation in spite of lactic acid increase is partly accounted for by the evident overventilation of the animals, but Ingraham and Gellhorn (5) have reported similar pH changes during anoxia with a constant rate of artificial respiration. The importance of blood flow as a regulator of cortical pH is undoubtedly due in part to the effect of blood flow on the rate of removal of carbon dioxide from the tissue.

Previous workers have given evidence that the phosphorylating glycolytic cycle, well known in muscle, can function in the brain *in vitro*. In this study the observed occurrence of phosphocreatine breakdown accompanying lactic acid formation during oxygen lack and the rapid resynthesis of phosphocreatine during recovery show that this mechanism is also operating in the brain *in vivo*. The findings reported here are very similar to those reported by others on muscle in the early stages of oxygen lack and of fatigue (6). Similar observations have also been made on peripheral nerve (7).

The rapid disappearance of lactic acid from the brain during recovery from anoxia seen in these experiments is most probably due to removal of the substance by oxidation, for previous studies have shown indirectly that the diffusion rate of lactic acid between blood and brain is slow (8, 9). It would appear likely that the level of lactic acid in the brain is regulated by metabolic conditions within the tissue, the formation and removal proceeding by a reversible reaction with the aid of the lactic dehydrogenase which is known to be present.

The electrical activity of the cerebral cortex is well known to depend upon the adequacy of its oxygen and its glucose supply. But the relation of intermediary metabolites to the electrical activity of the brain is unknown, and the present results do little to clarify the situation. If the oxygen supply is interrupted, the cortex becomes eletrically silent after a slight decrease of phosphocreatine has taken place and before adenylpyrophosphate has been altered at all. With oxygen present, greater changes

in phosphocreatine and lactic acid have at times been observed without loss of electrical activity. The conclusion is thus forced upon one that although breakdown of phosphocreatine does take place in brain as well as in muscle, the energy necessary for the maintenance of electrical activity of the brain cannot be obtained from the phosphorylating systems present, nor from anaerobic glycolysis by any route. It may well be that electrical activity is maintained by the oxidation of glucose through some pathway independent of the phosphate cycle.

SUMMARY

1. The cerebral cortex has been analyzed, after freezing *in situ* with liquid air, for the following constituents: lactic acid, inorganic phosphate, phosphocreatine, pyrophosphate and "hexose phosphates".

2. Injury or removal of one area of the cerebral cortex can result in marked chemical changes in adjacent and remote areas.

3. The concentrations of lactic acid and of inorganic phosphate are increased and that of phosphocreatine is decreased in conditions of anoxia resulting from breathing nitrogen or from reduction of cerebral blood flow. The two conditions differ in that the electrical activity disappears while the cortex is shifting in an alkaline direction in the first and disappears while the cortex is shifting in an acid direction in the second.

4. The electrical activity of the cortex is obliterated in conditions of anoxia before any detectable changes in the levels of adenylpyrophosphate or "hexose phosphates" take place.

5. During recovery from anoxic anoxia, lactic acid promptly decreases to its normal level in the brain and phosphocreatine is apparently resynthesized. The concentrations of inorganic phosphate and phosphocreatine tend to "overshoot" their original values, while the electrical activity is showing a rebound.

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THE RESPONSE OF THE CEREBRAL CORTEX TO LOCAL APPLICATION OF STRYCHNINE NITRATE[1]

J. G. DUSSER DE BARENNE, CLYDE MARSHALL, LESLIE F. NIMS AND W. E. STONE[2]

From the Laboratories of Neurophysiology, Neuro-Anatomy, and Physiology, Yale University School of Medicine

While the physiological effects of strychnine on the central nervous system are sufficiently understood to make it serviceable in many investigations, a satisfactory theory of how the chemical effect is mediated has yet to be deduced. This paper presents certain observations which should be taken into account in the construction of any theory of the chemistry of strychnine action.

PROCEDURE. Two types of experiment have been performed. In the first, strychnine was applied to a small area (2 by 3 mm.) of the sensori-motor cortex of a cat (0.5 per cent solution of strychnine nitrate) or of a monkey (3 per cent strychnine nitrate). The electrical activity of such a strychninized area was then followed continuously while the blood pressure or the oxygen content of the blood was varied.

In the second type, one hemisphere of a cat was painted with a 3 per cent solution of strychnine nitrate, and the pH (1) as well as the electrical activity studied. Twenty minutes later, while the strychninized cortex was showing the characteristic electrical activity designated as "spiking" by Dusser de Barenne and McCulloch (2), the treated area and the homologous contralateral hemisphere were frozen simultaneously *in situ* with liquid air. The tissues were examined chemically by procedures already described (3, 4).

Five cats and two monkeys anesthetized with nembutal and dial respectively were used for the blood pressure and low oxygen experiments, and each animal was subjected to repeated short periods of reduced blood pressure or lowered oxygen supply. The blood pressure was reduced by so adjusting an artificial respiration machine that increased air pressure in the lungs was maintained. The level of the blood pressure was indicated

on a mercury manometer connected to a femoral artery. The oxygen content of the blood was lowered by allowing the animals to breathe nitrogen containing small amounts of oxygen (0 to 4 per cent).

RESULTS. A short time after the local application of strychnine to the cerebral cortex the distinctive type of electrical activity (spikes) appeared. When the blood pressure was progressively reduced, the strychnine spikes decreased first in frequency and then in amplitude, finally disappearing altogether at blood pressure levels of 30 to 40 mm. of mercury. The "spontaneous" electrical activity was decreased by this procedure but was still present at a time when strychnine spikes were no longer seen. When the blood pressure was allowed to rise the spontaneous activity increased first; later the strychnine spikes reappeared and gradually returned to their former frequency and amplitude. Several of the cycles could be followed after a single application of the strychnine solution, showing that the initial disappearance was not simply a matter of the strychnine effect wearing off. Selected samples of the electrocorticogram obtained during one such cycle are contained in figure 1.

In one animal, a monkey, whose blood pressure was initially in the vicinity of 40 mm. Hg, strychnine spikes were not obtained following local strychninization until the blood pressure had spontaneously improved.

When the animals were made to breathe nitrogen containing small amounts of oxygen, the strychnine spikes were found to be more sensitive than the spontaneous activity to anoxia. The frequency of spiking decreased during the period of anoxia and finally the spikes disappeared at a time when the spontaneous activity was only slightly reduced in amplitude. When air was readmitted, the strychnine spikes returned, their amplitude and frequency increasing until they were again at their pre-anoxial level. It was possible to carry the animal through several such cycles following a single strychninization. In figure 2 are selected portions of a record of a single cycle.

Following the local application of strychnine, no pH changes have been observed at the site strychninized which could be ascribed with certainty to either the strychninization itself or to the intense electrical activity that follows. Similar findings with respect to pH have also been reported by Jasper and Erickson (5).

A comparison of the chemical analyses of a strychninized and an untreated hemisphere shows that there are no significant differences in the concentrations of inorganic phosphate, phosphocreatine, adenylpyrophosphate or "hexose phosphates" of the two areas. As can be seen in table 1, there may be a slightly greater concentration of lactic acid in the strychninized hemisphere, but the increase is so slight that a large number of animals would be necessary to establish the validity of the increase, if any.

DISCUSSION.   It is apparent from the results reported here that strych-
nine spikes are more susceptible to a reduced supply of blood or oxygen
than is the normal electrical activity.   The production of strychnine spikes
apparently will take place only if oxygen is present at a somewhat higher
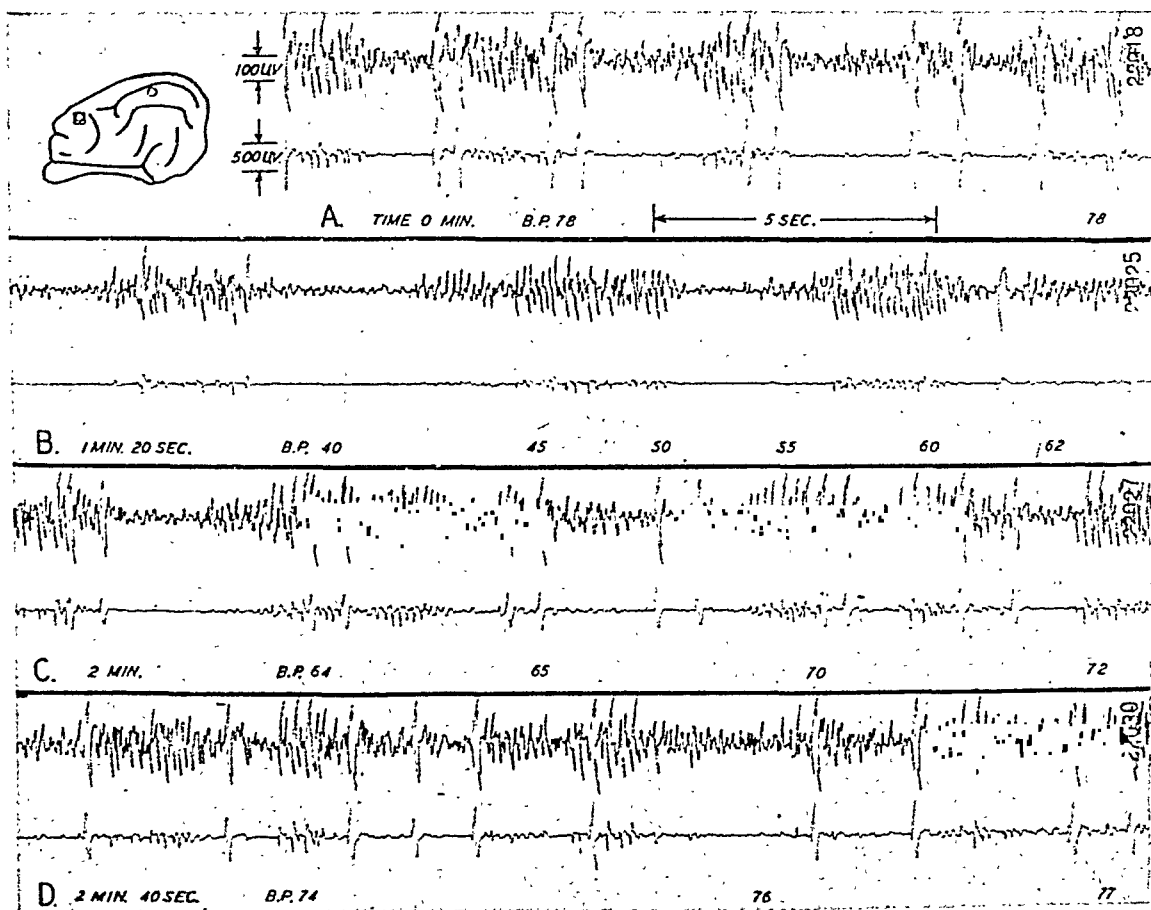
Fig. 1. The differential effects of low blood pressure on the "spontaneous"
electrical activity and the strychnine "spikes" of a nembutalized cat.   The two
recordings were taken from the indicated areas of the brain at different amplifications
in order to bring out the background activity in the upper record and the spikes in the
lower.   These records were obtained with a Grass electroencephalograph.   The time
and the blood pressures are recorded on the individual records.   Blood pressure was
reduced by raising the intrathoracic pressure.   □ in the inserted diagram indicates
the strychninized area.   O indicates the electrode placement.
     Note the disappearance of the strychnine spikes at low blood pressures in B and the
diminished amplitude during recovery in C and D while the background activity is
affected much less.

partial pressure than is necessary to maintain the "spontaneous" electrical
activity.
     It is somewhat surprising that such apparently intense electrical activity
can appear in one portion of the brain without producing more distinct
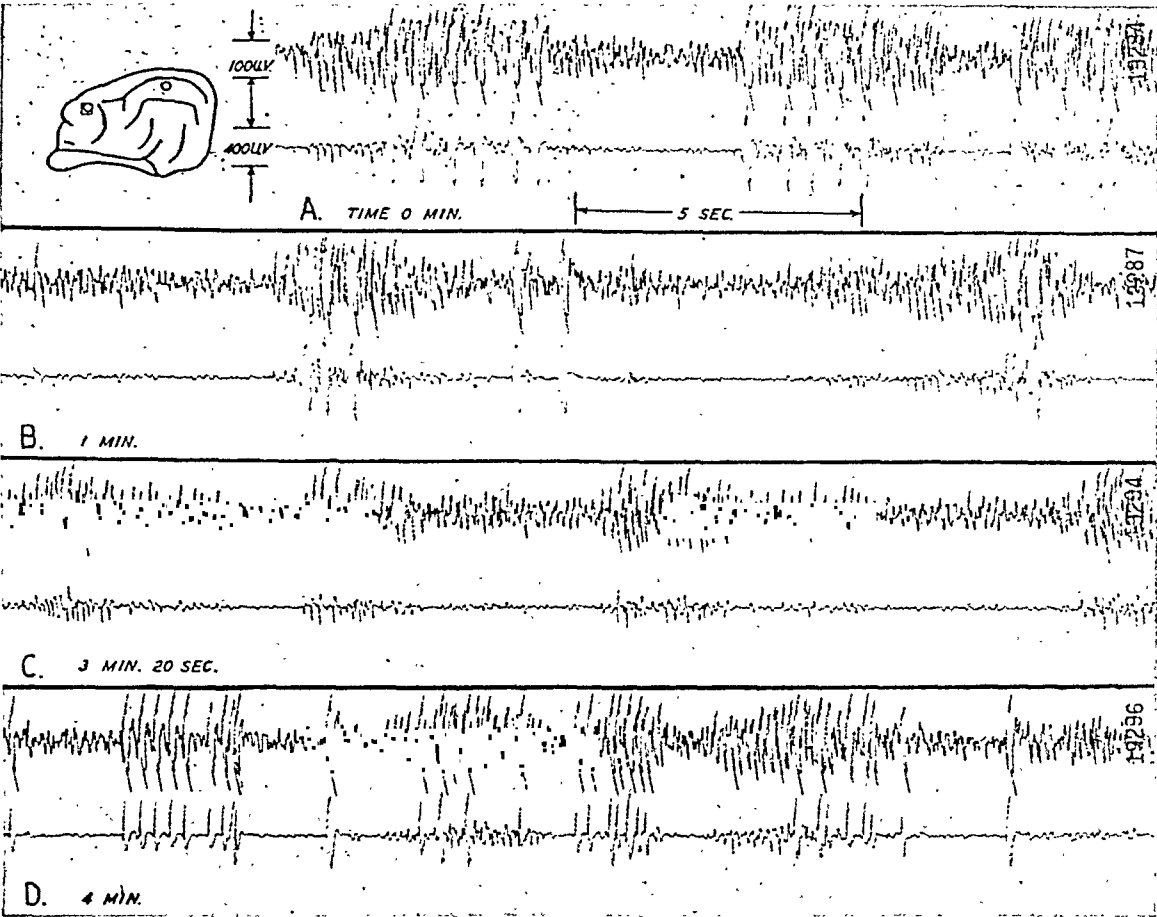
Fig. 2. The differential effects of low oxygen on the "spontaneous" electrical activity and the strychnine "spikes" in a nembutalized cat. Legends as for figure 1. In A the animal began breathing 4 per cent oxygen in nitrogen. In C the animal was returned to air.

Note decreased frequency of the spikes in B, the absence in C, and the return in D, while the background activity is hardly affected.

TABLE 1

Effects of local application of strychnine on pH, lactic acid and phosphates of the cortex

HEMISPHERE	pH		LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
	Before	After					
	Application of strychnine to one hemisphere						
			mgm. per 100 gm.	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*
Normal.......	7.20	7.18	20.2	11.1	14.6	11.0	10.5
Str...........	7.10	7.15	23.2	12.3	15.4	11.9	12.7
Normal.......	7.25	7.30	16.8	11.4	13.1	12.4	17.0
Str...........	7.15	7.17	20.3	10.0	12.1	11.0	18.7

chemical differences between that region and the untreated portion. The latter findings would be understandable, however, if the supposition is made that strychnine does not stimulate cells to greater activity, but rather synchronizes such activity as the cells possess. (See Bremer (6) and Gerard (7).) It might be supposed that if large numbers of cells were stimulated the products of their increased metabolism would modify the chemical pattern of the tissue, whereas if only synchronization of existing activity is effected, the chemical state of the tissue would remain essentially unaltered. This, however, can only be a supposition, for our present chemical methods are very crude and incomplete, and do not in any sense approach the recording of electrical activity in delicacy.

SUMMARY

1. Strychnine "spikes" are more sensitive to anoxia than is the "spontaneous" electrical activity of the cerebral cortex.

2. The application of strychnine to the cerebral cortex produces no significant changes in the pH or in the concentrations of inorganic phosphate, phosphocreatine, adenylpyrophosphate or "hexose phosphates".

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MEASUREMENTS OF INTRAMYOCARDIAL PRESSURE[1]

DONALD E. GREGG and RICHARD W. ECKSTEIN

From the Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio

Using the hypodermic manometer Gregg, Eckstein and Fineberg (1) showed that left ventricular pressure exceeding aortic pressure (as reported by Hamilton, 2) is recorded only when the needle tip does not lie free in the ventricular cavity. Since, by inspection, it was known in most instances that the needle opening was in a myocardial pocket of fluid it seemed a possible means of quantitating intramural pressure. Accordingly, these experiments were extended and attempts were also made to measure myocardial pressure through imbedded arterial segments (unpublished observations). Generally such pressure pulses exceeded the aortic systolic, but due to technical difficulties these measurements were not believed to demonstrate crucially that intramyocardial pressure exceeds ventricular during systole. Johnson and DiPalma (3) by use of imbedded arterial segments have recorded pressure pulses from the myocardium exceeding aortic pressure and believe they are an exact measure of intramyocardial pressure. Since these experimental findings are interpreted differently we have reinvestigated the subject.

METHODS. The chest and pericardium of dogs were opened under sodium pentabarbital anesthesia and under artificial respiration. The blood was made non-coagulable by a mixture of heparin (100 units per kilo) and pontamine fast pink (200 mgm. per kilo). Attempts were then made to evaluate intramyocardial pressure in the following ways: 1. The systolic and diastolic pressures in a vessel segment closed at its peripheral end and imbedded in the left myocardial wall were determined in a manner similar to that of Johnson and DiPalma (3) and compared with the existing aortic or left ventricular pressure. 2. The phasic rates of flow through such imbedded vessel segments were determined by the orifice meter (Gregg and Green, 4) with blood led from the aorta or left ventricle, or with blood or Locke's solution led from the constant pressure meter (Green and Gregg, 5). 3. Pressures were recorded from intramyocardial

pockets of mineral oil, glycerine, Locke's solution and heparinized blood placed at varying depths.

RESULTS. *Recordings from imbedded vessel segments.* A pressure manometer was attached to one end of a segment by a cannula, the tip of which was generally, although not always, buried in the myocardium, while the other end of the imbedded segment was ligated at the epicardial surface. Pressures were then recorded under various distending intra-vessel pressures. These experiments confirm the findings of Johnson and DiPalma (3) that the pressure pulse generally exceeds the aortic or left ventricular pressure. However, when the aortic pressure is essentially constant at 90/74 mm. Hg an increase in the diastolic internal distending pressure from 84 to 204 mm. Hg causes a very large increase in the pressure pulse from 120 to 196 mm. Hg (fig. 1-A and B). Similar results were obtained with rubber tubing of comparable wall thickness.

Further massive augmentation of the vessel segment pulse (up to four times the left ventricular pressure) is obtained by the simple expedients of pulling the thread attached to the peripheral end of the segment so that it is maintained at an increased length (records not shown) or by elevation of blood pressure through mechanical constriction of the aorta (fig. 1-C vs D). Such values greatly exceed any normal expectancy for intra-myocardial pressure.

In some experiments, to minimize possible movements of the vessel segment, it was pulled over and tied to a small, rigid, fenestrated metal tube which was closed and tapered peripherally and attached to the manometric system. This procedure did not alter the pressure relationship recorded in the myocardium and aorta.

Blood flow through an imbedded vessel segment. If intramyocardial pressure exceeds aortic systolic, as the preceding experiments suggest, then blood piped from the aorta under its normal pressure head into a deeply imbedded carotid segment (open at its peripheral end) should cease to flow during at least part of systole. Actual measurements of such phasic flow by the orifice plate meter (Gregg and Green, 4) interposed between the segment and the aorta may show at times a sizable systolic flow. Figure 1-E is a record from an experiment in which the minimal rate of systolic flow approximates 12 cc. per minute. Similarly, when Locke's solution held under a slowly declining pressure is led through the segment from the constant pressure meter of Green and Gregg (5) there may be a systolic flow of approximately 14 cc. at infusion pressures 8 mm. Hg less than aortic diastolic (fig. 1-F). These experiments indicate that, as measured by this method, the systolic resistance to coronary flow may be considerably less than aortic systolic pressure.

Recordings from myocardial fluid pockets. These pockets were produced by injecting oil, Locke's solution or heparinized blood under pressure into

the muscle wall of the left ventricle.   The pulses were recorded from these
pockets with hypodermic needles or fenestrated and tapered cannulae to
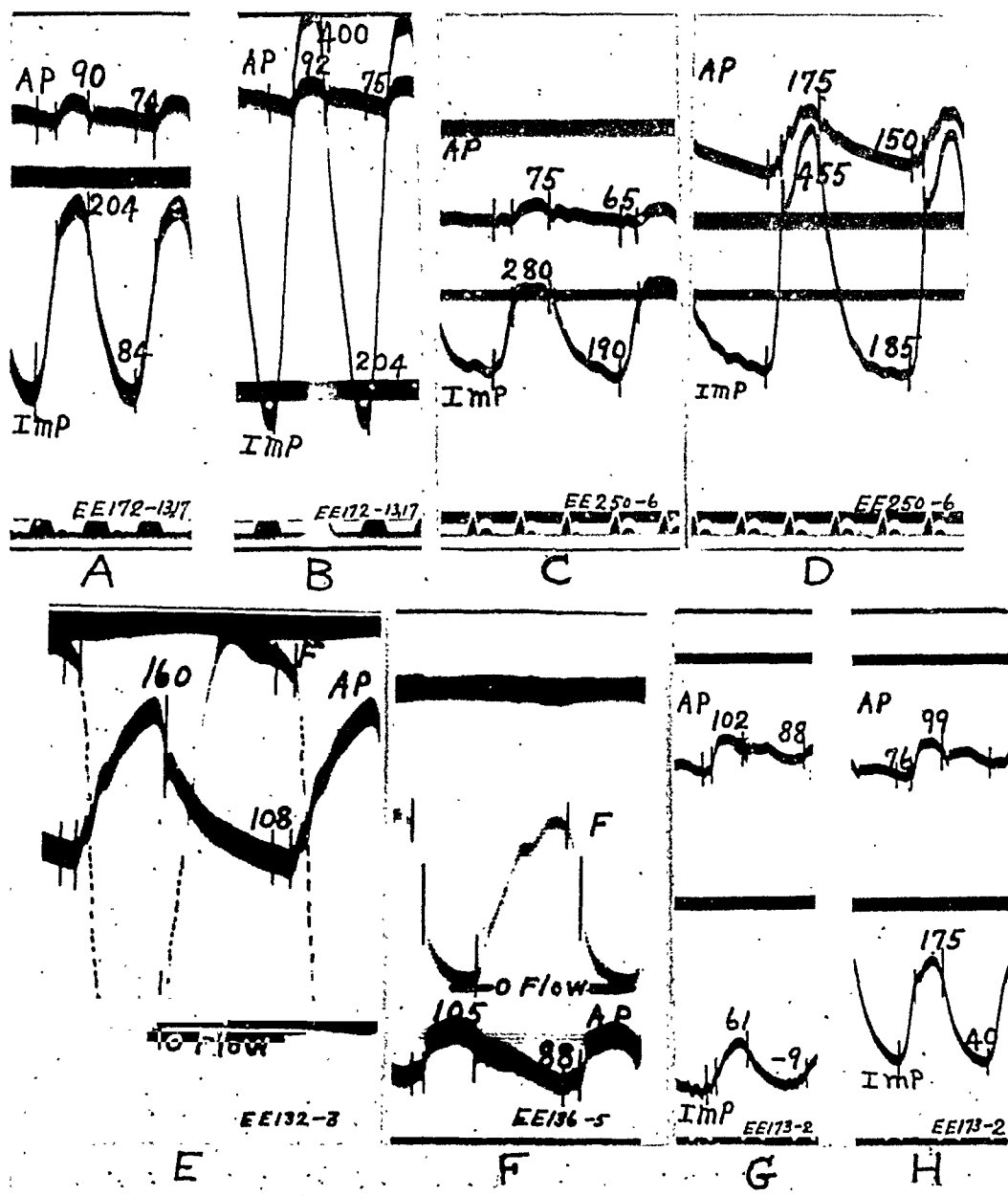
Fig. 1. A, B—records illustrating pressure pulses recorded from arterial segment
imbedded in left ventricular wall at different diastolic segment pressures.   C, D—
records showing comparative effect of aortic constriction on aortic blood pressure
and intrasegment pressure.   E, F—records using orifice plate meter showing phasic
blood flow through artery segments imbedded in myocardium of left ventricle (E with
blood from carotid; F with Locke's solution from infusion chamber).   G vs. H—
records of pressure taken from myocardial pockets of blood in left ventricular wall
and showing the effect of elevation of diastolic pocket pressure.   AP—aortic pres-
sure; IMP—intramyocardial pressure taken through vessel segment or fluid pocket.
F—coronary inflow calibrated at top of light beam.   Time—$\frac{1}{5}$ second.

prevent any possibility of occlusion. Figures 1-G and 1-H show that, as the diastolic pressure in a myocardial pocket of heparinized blood is increased during diastole from -9 to 40 mm. Hg, the resulting pulse rises from 70 to 135 mm. Hg, with a fairly constant aortic pressure. The same results were obtained with the other substances. In these experiments it was never possible to demonstrate the tremendous excesses of pressures recordable from the myocardium with vessel segments.

DISCUSSION. From the foregoing experimental work it is evident that pressure pulses in excess of aortic or left ventricular pressure can be recorded from well imbedded vessel segments or from myocardial pockets of fluid. However, since with vessel segments *1*, the intramyocardial pressure thus recorded may be so high (two to four times the aortic pressure) and may undergo further massive augmentation when the segment is stretched or the blood pressure is raised; *2*, a systolic flow of blood may occur through such a segment when the central end of the segment is connected to the aorta for blood source, and *3*, in myocardial pockets the pressure pulse may increase greatly with the diastolic distending pressure, it is not believed that such methods faithfully record the true pressure existing in the intramyocardial space.

Experiments were then devised to find out if possible what artefacts contributed to the production of these myocardial pressure curves. It was felt that the vessel segment per se or movements and distortion of the segment induced either by fluid movement or myocardial impact might affect the pressure transfer. Accordingly, the hydrodynamic principles involved in the transmission of pressure through the walls of elastic tubes were studied *1*, with a mechanical model; *2*, with pressures recorded from small rubber bags, vessel segments and rubber membranes inserted into the ventricular chamber, and finally *3*, with pressures recorded from the segments protected by a fenestrated metal cap or by a fenestrated and retractable metal sleeve.

Pressure transfer in a schema. Two possibilities suggest themselves as to why the vessel segment per se might limit pressure transfer through it. 1. In such a recording system, which is not isometric, any membrane exerting tension and interposed between a manometer and the pressure source to be measured prevents 100 per cent pressure transfer. This is predicted by the equations:

$$(A) \quad \text{Pressure transfer} = \frac{-T}{r} + \frac{T'}{r'} + P'$$

in which P' is the applied external pressure, T and T' are the wall tensions and r and r' are the radii. Therefore, pressure transfer is complete only if $\frac{T}{r}$ equals $\frac{T'}{r'}$. But

$$(B) \quad \frac{T}{r} = A_0(1 - r_0/r) \quad \text{and} \quad \frac{T'}{r'} = A_0(1 - r_0/r')$$

in which r_o is the resting radius and A_o is a constant.   Hence, since r' is less than r, $\dfrac{T}{r}$ will be greater than $\dfrac{T'}{r'}$ and pressure transfer will not be complete.   2. The volume/pressure ratio in a vessel segment is not constant but decreases (especially rapidly at higher pressure levels).   This must mean that pressures transfer into the segment from an outside source, for equivalent pressures applied, becomes progressively less at higher distending pressures.

These predictions of less than 100 per cent transfer are demonstrated experimentally in the data in figure 3 obtained by use of the schema in figure 2.   The schema consists of a rubber bag, tube or vessel segment, C, placed in a Lucite chamber, A.   The elastic structure and chamber were filled with Locke's solution or blood, and various pressures placed in both
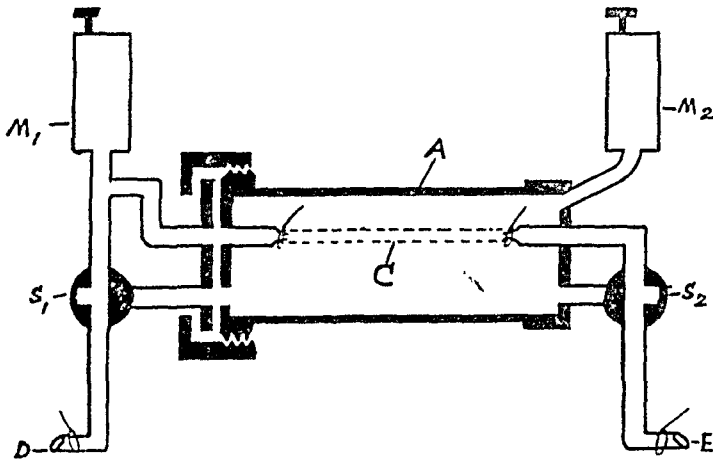
Fig. 2. Apparatus to determine extent of pressure transfer through vessel segments, rubber tubes and membranes under static and dynamic conditions.   A—Lucite chamber; C—artery segment; M_1 and M_2—pressure manometers to determine pressure inside and outside segment; S_1 and S_2—stopcocks to direct pressures and blood flow through segment and chamber.   D and E—cannulae.

were varied independently by turning appropriate stopcocks.   The resulting pressure transfer was measured by Gregg pressure manometers (1, 6) M_1 and M_2.   In any one experiment in which the segment was filled with fluid and the external pressure raised in successive increments the extent of such transfer was roughly the same over wide ranges of internal and external pressures, but in different experiments pressure transfer varied from 80 to 100 per cent.   (See plot in fig. 3.)   The pressure loss is maximal at the higher levels of segment inflation and can be significant at all pressure levels.

When the external pressure in the chamber was created by a pump system (producing a pulse pattern approximating a left ventricular pressure curve) pressure transfer could be augmented greatly by so directing the chamber flow that the artery segment moved.   In figure 3 points in areas F and G

in which segment moved are to be compared with points in areas D and E in which segment did not move. The actual increase in pressure transfer is directly related to the extent of artery movement and inversely related to the diastolic inflation pressure. Here at a constant diastolic inflation pressure of 152 mm. Hg segment movement increases pressure transfer from 85 to 120 per cent (areas D versus F).
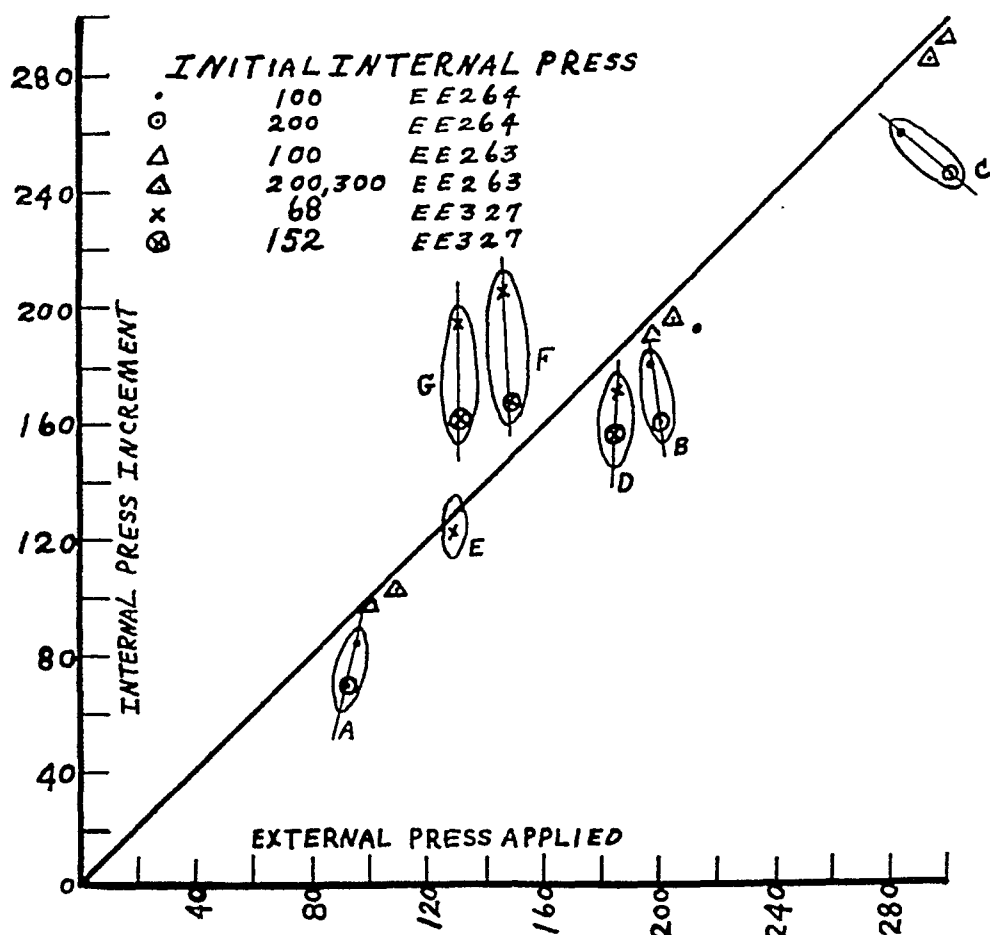
Fig. 3. Graph showing extent of pressure transfer through vessel segments using apparatus of figure 2. Initial internal pressure is intrasegment pressure before external pressure applied. Abscissa—mm. Hg external pressure applied. Ordinate—mm. Hg increase in intrasegment pressure due to applied external pressure. Solid line—theoretical curve for 100 per cent transfer.

Finally, when the chamber and segment are attached to the carotid artery of an anesthetized dog so that the blood pressure is transmitted to the chamber the pulse values recorded simultaneously from the external chamber and segment may be identical or may differ. An example of 93 per cent transfer is illustrated in figure 4-A. As a result of vagal stimulation the carotid pulse pressure is 95 mm. Hg (112/17), while the intrasegment pulse pressure is 88 mm. Hg (225/137).

Recordings from the ventricular cavity. To test further the accuracy of vessel segments as recording devices and under conditions which approximate those in the myocardial wall, the segments were attached to the manometric system by a metal cannula and then pulled into the left ventricular cavity (generally through the apex) by a needle and thread so that the whole segment was entirely free in the cavity. Its position was verified at the close of the experiment. If accurate, such closed segments should, at maximal inflation, give a pulse similar in contour, magnitude and timing to that obtained from the ventricle through an open cannula
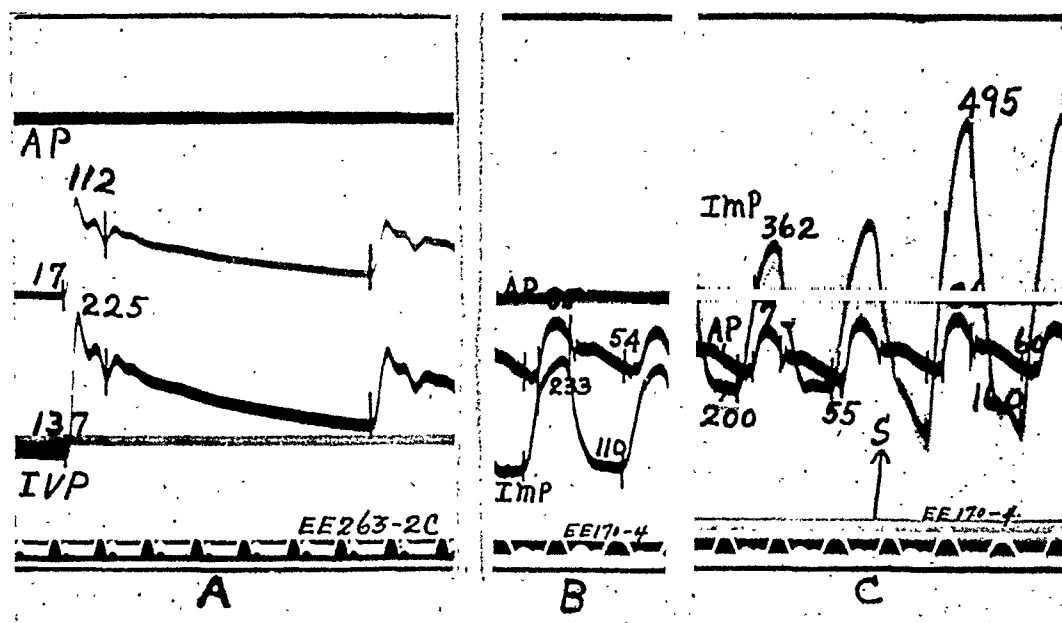
Fig. 4. A—record showing relationship between normal carotid pressure pulse, A.P., recorded from chamber A and pulse, I.V.P., after transfer through a carotid segment, C, in figure 2. B—record showing relationship between pressure pulse recorded from a closed artery segment in left ventricular cavity and the aortic pressure. C—record showing further augmentation of pulse in B by increasing diastolic intrasegment pressure and by stretching segment at S. AP—aortic pressure; IMP—intravessel pressure in left ventricular cavity; IVP—intravessel pressure in Lucite chamber; S—time at which vessel stretched; time—$\frac{1}{5}$ second.

or needle. In records figure 4-B and C elevation of the intrasegment pressure from 110 to 200 mm. Hg during diastole increases the pulse from 123 mm. Hg (already greater than aortic systolic) to 162 mm. Hg, while the aortic pressure remains at approximately the same pressure level. If now the aortic pressure is elevated by mechanical constriction of the aorta the increase of intrasegment pressure may double the change in aortic pressure (records not shown). In either instance, if the segment is now stretched by a cord extending through the ventricular wall and attached to its peripheral end, such pressure pulses can be increased still further.

In the last two pulses, figure 4-C, such a procedure augments the pulse from 162 to 335 mm. Hg. The maximum pressure thus far recorded from the left ventricular cavity by a closed vessel segment has been five times the ventricular pressure as registered through an open cannula.

Recordings from protected segments. To reduce the errors in pressure transfer thus revealed as caused by movement and changes in shape of the vessel segment the vessels were protected by placing over them loosely fitting fenestrated metal caps or metal sleeves, either of which could be removed as desired. Figure 5, A versus B, illustrates the effect on the pressure pulse of covering of the segment in the left ventricular cavity with a metal sleeve. Placement of the sleeve in B changes the pulse contour
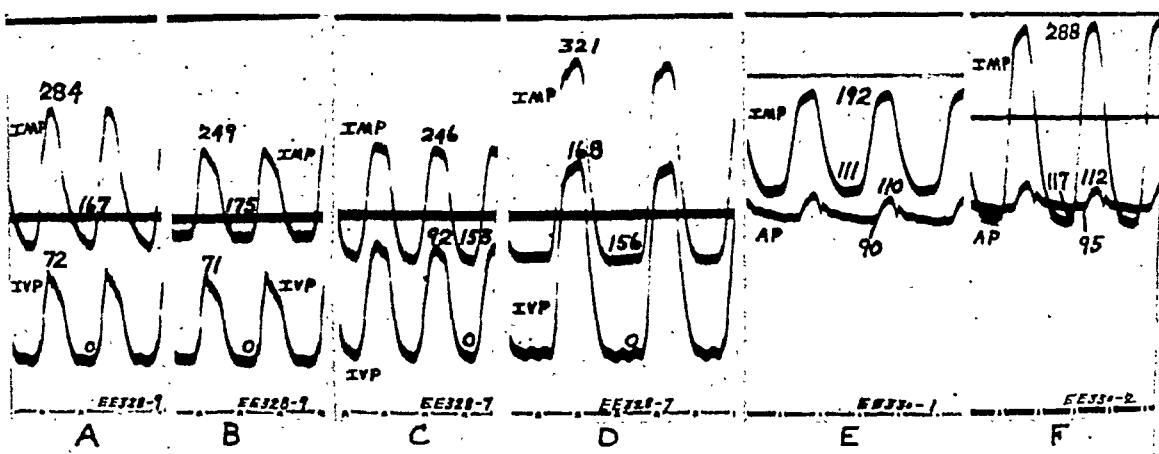
Fig. 5. A, B—records showing depression of intrasegment pressure in left ventricular cavity following addition of a protective fenestrated sleeve in B. C, D—records illustrating the comparative effects of mechanical constriction of the aorta on left ventricular pressure and intrasegment pressure when the segment is protected and in the left ventricular cavity. E, F—same as C, D but protected and unprotected segment in left ventricular wall. AP—aortic pressure; IVP—intraventricular pressure; IMP—intravessel segment pressure. Time—$\frac{1}{5}$ second.

to the ventricular pattern and reduces the pulse pressure value from 117 to 74 mm. Hg or to a value agreeing with the ventricular. One hundred per cent transfer may also occur when the intravessel distending diastolic pressure varies from 0 to 300 mm. Hg and when the blood pressure is raised by aortic constriction or synephrine injection (cf. fig. 5-C, D). Similarly, when a vessel segment protected by a sleeve is imbedded parallel to the left descendens and approximately one-half the depth of the myocardium, removal of the sleeve in F as compared to E, changes the pulse contour and greatly increases the pressure pulse value from 81 to 171 mm. Hg.

Protection of the segment thus reduces the error, and the agreement between the intraventricular pressure and intra-vessel pressure in figure 4

suggests that covered segments may give essentially correct values. However, such a possibility is remote. In the schema and in the ventricular cavity the pulse in the protected segment differs somewhat from the ventricular pressure curve, and predictions based on calculations and verified in many experiments (cf. fig. 3) indicate that pressure transfer can be incomplete. Finally, the fact that the pressure pulse recordable from an intramyocardial pocket of fluid or blood, although considerably greater than ventricular, increases progressively upon raising the pocket pressure, suggests that such a pulse is partially a function of the degree of stretch of local muscle fibres induced by the applied internal pressure. This error would presumably be present also in the imbedded vessel segments. Since these errors can not be removed the method of myocardial pockets of fluid or imbedded segments can not be used in a quantitative manner. However, since all these errors except the last can be quantitated for each segment in a schema the possibility remains for the use of either method in acute experiments as a rough index of directional changes in intra-myocardial pressure.

SUMMARY

Pressure pulses registered from myocardial pockets of fluid or by means of vessel segments imbedded in the myocardium of the left ventricle generally exceed by considerable amounts the aortic pressure simultaneously recorded. However, such pressures are in part artefactually produced and hence do not crucially demonstrate that intramyocardial pressure exceeds left ventricular pressure during systole. Our reasons for this belief are as follows: 1. The pressure pulse from an intramyocardial pocket of fluid increases progressively to values greater than aortic pressure upon raising the diastolic pressure in the pocket of fluid, and hence it is probably a function of the degree of localized muscle stretch induced by the applied internal pressure. 2. When blood from the aorta flows through an imbedded segment a systolic flow may occur. 3. Pressures recorded from closed vessel segments in the left ventricular wall or cavity may have two to four times the ordinate value of the intraventricular pressure, although the usual values are somewhat lower. 4. Protection of the segment in the cavity or wall by a loose fitting fenestrated cap or retractable metal sleeve reduces the recorded pressure pulse in the left ventricular cavity, in many experiments, to values approximating aortic pressure, and in the myocardium to values somewhat less than aortic pressure. 5. Theoretical and experimental evidence is given to show that pressure transfer through protected vessel segments is generally not complete. 6. However, pressure transfer exceeds 100 per cent when there is movement and distortion of the segment as a result of turbulent flow or mechanical impact. 7. Although the use of vessel segments may give directional changes, ex-

act quantitation of intramyocardial pressure is not yet believed to be a reality.

The authors wish to express their appreciation to Doctors W. Pritchard, T. Steege, and R. E. Shipley for their assistance in some of these experiments.

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THE VIABILITY OF SPERMATOZOA IN THE ABDOMINAL EPIDIDYMIS AND THE FAILURE OF MOTILE SPERMS TO FERTILIZE OVA

S. A. ASDELL and G. W. SALISBURY

From the Animal Nutrition Laboratory and Department of Animal Husbandry, Cornell University, Ithaca, New York

Since the experiments of Hammond and Asdell (1926) upon the viability of spermatozoa in the male and female tracts, a good deal of work has been done which shows that the results obtained by them are of wide application among mammals. Bats alone do not seem to conform, as Guthrie (1933) has shown. Hammond and Asdell found for the rabbit that the fertility of sperm retained in the scrotal epididymis was 100 per cent up to 21 days, from 21 to 30 days it was 36 per cent, from 31 to 40 days, 20 per cent, and after this time the sperm were infertile. They retained their motility in one case, however, for 60 days. Probably part of this fertile time is taken up in the maturation of the younger sperm in the epididymis, so that the extreme time includes both the maturation period and the time during which the mature sperm retain their fertilizing ability. In this connection Young (1931) has found for the guinea pig an increase in the fertility of spermatozoa taken from the epididymis as the vas deferens is approached. This may account for 25 or more days of the life of the sperm of the guinea pig (Toothill and Young, 1931).

Young (1929) found that guinea pig spermatozoa remained fertile for up to 35 days when they were kept in the epididymis. They retained their motility, however, for 59 days. White (1933) found a fertile life for rat sperm up to 21 days, with a retention of motility for 42 days.

As the fertile life of rabbit spermatozoa in the female tract is limited to 30 hours (Hammond and Asdell, 1926), it is interesting to decide whether this reduced life is due to the higher temperature of the abdomen or to the motility of the sperm in the female tract, to mention but two of the possible reasons for the difference. Knaus (1932) has given data bearing on this point. He found that spermatozoa in the isolated abdominal epididymis were fertile for a maximum of 4 days, while in the isolated scrotal epididymis they retained their fertility for 12 days. The difference between the latter result and that of Hammond and Asdell, who found a fertile life of 40 days, is probably due to an earlier exhaustion of the supply

of sperm. Knaus was mating his rabbits daily, so that the one of three rabbits that maintained fertility to the twelfth day had mated seven times; when he was tested on the twenty-ninth day he was sterile. Motility was preserved in the abdominal isolated epididymis for a maximum of 12 days. He also determined the duration of fertility in the cryptorchid rabbit, in which the connection between the testis and the epididymis was undisturbed. His data are rather scanty and his rabbits were each mated several times. The maximum duration of fertility by this method was 7 days in one rabbit of three; at 6 days the fertility was two rabbits of three; while at 8 days no rabbit of three was fertile. He found motility in one rabbit of four at 14 days and in no rabbits of two at 15 and 16 days. From Knaus' data one concludes that the increased temperature without motility is instrumental in reducing both the fertile and potentially motile life. The duration of fertile life is reduced from 40 to 4 days by the increase of temperature in the absence of motility, while potential motility is reduced from 60 to 12 days' duration. These results were from the isolated epididymides. The reduction in the duration of fertility in spermatozoa from the testis was not quite so great. We have examined testes after a stay of 24 hours in the abdomen and have found that spermatogenesis has already ceased, so that in none of this work does the replenishment of the spermatozoa arise as a complication. Increased temperature, therefore, brought the fertile life down from 40 to about 7 days, and motility decreased this further to 30 hours.

We have now obtained more precise data on the duration of fertility and motility of spermatozoa in the testis and epididymis, and have determined the cause of the lack of connection between fertility and motility.

EXPERIMENTAL. We have used for the purpose of this work a number of male rabbits of Flemish Giant type of proven fertility and one year old or a little more. We have tried to use them as far as possible at one year of age. The method employed was to anchor the testes to the ventral abdominal wall with a loose suture through the fat body surrounding the caput epididymidis. Care was taken not to interfere with the blood supply in any way. The rabbits were then kept isolated until the day for testing, when they were given one or two matings. Later they were killed for motility studies. There was thus no possibility of semen exhaustion through previous matings. The results are summarized in table 1. Only bucks which actually mated are included in this table.

Our results agree closely with those of Knaus. We find that the extreme fertile life of the spermatozoa in the abdominal testis is 8 days compared with the 7 days found by him. Of 10 bucks mated to 15 does at 9 days, not one was fertile, and the same lack of fertility was obtained with 7 bucks mated to 14 does at 10 days. It may be added that we found no evidence of a reduction in the litter size in the fertile matings. A

'large number of spermatozoa must have retained their fertility for a period and then lost it quite rapidly.

Table 2 gives the motility of the spermatozoa in these and other bucks used in this work. The procedure was to mince the epididymides and to add Ringer solution and examine on a slide or as a hanging drop. Each record represents a separate buck.

TABLE 1

Fertility of rabbits with abdominal testes

	DAYS IN ABDOMEN									
	1	2	3	4	5	6	7	8	9	10
No. of males tested.........	3	6	2	5	6	0	7	6	10	7
No. of matings..............	3	6	2	5	6		7	11	15	14
No. of fertile matings.......	3	4	1	4	3		1	3	0	0
No. of fertile males.........	3	4	1	4	3		1	2	0	0
Litter size of fertile matings..	6.3	5.75	3.0	6.75	6.3		7.0	7.0*	0	0

* Average of two litters, the other litter was partly destroyed by the doe.

TABLE 2

Sperm activity in samples from the epididymides in the abdomen

					DAYS							
1	2	3	4	5	6	7	8	9	10	13	14	15
T	T	0	M	T			T	F	S	0	0	0
M	M	M	M	M			T	F	T	0	0	0
M	M	M	M	S			F	S	T		F	0
M	M	M	M	0			T	F	F		0	0
M	M		0	T			T	T			0	0
M			M	T			T	S				0
M				T			T	T				0
				M				F				0
				M	.			F				0
				F				F				0
												0
												0

T = tremendous motility. M = motile. S = low motility. F = feeble motility. 0 = no motility.

It will be seen that motility ceased at about the fourteenth day. Twelve rabbits were tested on the fifteenth day without the detection of any motility. Only one specimen of five showed feebly motile spermatozoa at 14 days, so that this may be regarded as the extreme of motility. It was noted that the degree of activity of the individual spermatozoa and the number of motile spermatozoa agreed very closely. From table 1 it may

be seen that 4 bucks were sterile at 8 days. Of these, 3 had sperm with tremendous motility and 1 with feeble. This is additional evidence that fertility and motility are not parallel phenomena and that the lack of fertility at the extreme range of fertility is not due entirely to lack of evident vigor of the spermatozoa.

In order to decide why the motile spermatozoa did not fertilize eggs, 11 bucks with testes in the body cavity for 10 days were mated with does. The does were killed 9 or more hours after mating. Their reproductive tracts were searched for spermatozoa and for eggs. The results of the search were confirmed or corrected by sectioning parts of the oviducts. In two rabbits fertilization occurred, and examination of the bucks showed that the tail of the epididymis in each was in the inguinal canal. These records were rejected for this purpose. Records for the other 9 bucks and for the does follow:

1. Doe killed at 10 hours, sperm in the vagina and uterus, none in the oviducts.
2. Doe killed at 10 hours, no sperm found anywhere. In this case the epididymides were devoid of spermatozoa when the buck was killed at 15 days.
3. Doe killed at 10½ hours, a fair number of sperm in the vagina, a few in the uterus, none in the oviducts.
Doe killed at 12 hours, no sperm in the vagina, a few in the uterus, none in the oviducts.
4. Doe killed at 24 hours, a few sperm in the vagina, none in the uterus, none in the oviducts. Unfertilized eggs found.
5. Doe killed at 9 hours, no sperm found anywhere.
Doe killed at 24 hours, a few sperm in the vagina, a few in the uterus, none in the oviducts, 1 unfertilized egg found.
6. Doe killed at 12 hours, no sperm found in the vagina, a few in the uterus, none in the oviducts.
7. Doe killed at 16 hours, no sperm found anywhere, unfertilized eggs found. •
Doe killed at 42 hours, no sperm or eggs found anywhere. The doe had ovulated.
Dead spermatozoa were found in the epididymis at 15 days.
8. Doe killed at 16 hours, sperm found in vagina and uterus, none in the oviducts.
Doe killed at 48 hours, no sperm found anywhere, degenerating eggs found.
9. Doe killed at 16 hours, no sperm found anywhere, degenerating eggs found.
Doe killed at 48 hours, no sperm found anywhere, degenerating eggs found.
Dead spermatozoa found in the epididymis at 15 days.

No motile sperm were found in any of the females. From these results it is concluded that a few of the bucks may have failed to fertilize the does because no live sperm were present at the time of mating. This might apply to bucks 2, 7 and 9. For the remaining 6 bucks spermatozoa were found in the female tracts as far up as the uterus, but none in the oviducts and none were motile at the time of examination. In contrast, the two bucks with epididymides in the inguinal canal may be cited. Both these bucks fertilized eggs.

10. Doe killed at 10 hours, motile sperm in the uterus, non-motile sperm in the oviducts.

Doe killed at 24 hours, non-motile sperm found in the oviduct. Fertilized eggs found.

11. Doe killed at 14 hours, motile sperm found in uterus, no sperm in the oviducts.

Doe killed at 48 hours, non-motile sperm found in the oviducts. Fertilized eggs found. `

Evidently the bucks with live spermatozoa were sterile because the sperms were able to travel as far as the uterus but lacked both the ability to travel further and the power to survive until the eggs were shed. This point now seems to be well established.

SUMMARY

The testes of adult male rabbits were anchored in the abdomen for varying periods. Spermatogenesis ceased within 24 hours. The extreme fertile life of sperms was 8 days for 2 males in 6 tested at this time. At 9 days none in 10 was fertile. No effect was observed on litter size at 8 days. Motility ceased at 14 days. Motile but non-fertile sperm failed to reach the oviduct or to survive to ovulation time after mating the bucks.

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THE EFFECT OF ETHER ANESTHESIA ON THE PLASMA VOLUME OF CATS

C. LOCKARD CONLEY

From the Department of Physiology, College of Physicians and Surgeons, Columbia University

The cat has often been employed in experimental studies of anesthesia, and many of the reactions of this animal to ether are well known. However, no previous report has been made concerning the effect of ether on the plasma volume in this species. It has been known for a long time that in dogs hemoconcentration regularly accompanies anesthesia with ether, and McAllister's (1937–1938) careful studies with the dye method showed that this is associated with and partly caused by an actual decrease in plasma volume, a fact subsequently confirmed by Bollman et al. (1938). Far less complete investigations in man indicate that ether anesthesia is associated with a much smaller reduction of plasma volume than occurs in the dog (Gibson and Branch, 1937; Ragan et al., 1939), but all of these determinations were complicated by the simultaneous use of other drugs or anesthetic agents. In a recent paper Barbour (1940) mentions two experiments on rabbits in which etherization was not attended by hemoconcentration, suggesting that no decrease in plasma volume occurred in these animals.

METHODS. Fifteen experiments were performed on 13 normal adult male cats. The animals were given no food for 16 hours prior to experiments but were permitted water ad libitum. During the period of observation they were placed on their backs on a board and restrained by the application of loose ties to the legs. With gentle handling it was found possible to keep unanesthetized cats thus lying on their backs for long periods without struggling. Some cats actually purred throughout the period preceding anesthesia.

Plasma volume determinations were made on the unanesthetized animal with the blue dye T-1824 (Gregersen et al., 1935–1939), the procedure being essentially the same as that described by Hamlin and Gregersen (1939). The medial aspects of both hind legs were shaved, and the dye was injected into a femoral vein through a 24-gauge hypodermic needle. At 10 to 20 minute intervals 1.5 cc. blood samples were drawn without stasis from the opposite femoral vein. After a control period averaging

100 minutes etherization was started.   The cat remained on the board, and ether was administered from a cone applied over the nose and mouth. By starting with low concentrations of ether in the cone, induction was accomplished with a minimum of struggling.   Deep anesthesia was maintained for a period varying from 30 to 80 minutes.   Blood samples were taken at regular intervals during the period of anesthesia, and changes in plasma volume were estimated from alterations in the disappearance curve of the dye.   The serum protein concentration of each blood sample was estimated from the refractive index of the serum.   In 4 experiments the serum protein concentration was also calculated from the serum

TABLE 1

CAT	DATE	WEIGHT	PLASMA VOLUME		DURA-TION OF ANES-THESIA	MAXIMUM PER CENT CHANGE IN		
						Plasma volume	Serum protein	Hemato-crit
		kgm.	*cc.*	*cc./kgm.*	*min.*			
1	2/18/38	2.95	138	46.8	30	0	0	
2	6/ 2/38	3.92	158	40.3	40	+6.4	−7.7	
3	6/ 7/38	3.36	164	48.8	30	0	0	
4	6/10/38	3.05	156	51.2	30	0	0	
	6/27/38	2.90	158	54.5	40	0	0	
5	6/15/38	3.16	208	65.8	60	0	−6.0	
6	6/17/39	3.29	133	40.4	45	0	0	
7	6/20/39	3.00	136	45.3	30	0	0	
8	6/23/39	3.30	137	41.5	80	0	0	
9	6/24/39	3.35	115	34.3	75	0	−6.5	0
10	6/27/39	3.30	132	40.0	50	0	0	+2.5
11	6/28/39	3.27	132	40.4	65	+5.5	−5.0	0
12	6/21/39	3.40	190	55.9	60	0	0	
	7/28/39	3.00	142	· 47.3	60	−7.4 to +8	+4 to −8	+4.7
13	12/20/39	3.86	173	44.8	50	0	0	0
Mean..................			151.5	46.5				

specific gravity determined by the falling drop method.   The values obtained by the two·methods were in close agreement.   In 5 experiments hematocrits were determined on heparinized blood using 1 cc. Wintrobe tubes centrifuged for 45 minutes at 3000 rpm.

RESULTS.   The results of the 15 experiments are recorded in table 1. In 12 experiments the dye curves revealed no change in the plasma volume during anesthesia, and in all except two of these the serum protein concentration remained constant throughout the procedure.   In the two exceptions (cats 5 and 9) there was a progressive fall in serum protein, but in one of these the decrease began before anesthesia was started.

. Three cats showed alterations in plasma volume during etherization,

coincident with the beginning of anesthesia. Two of these animals gave definite increases in the volume of plasma. In one the increase was 6.4 per cent above the pre-anesthetic value and persisted for 40 minutes, the duration of the experiment. Another cat showed an increase in plasma volume of 5.5 per cent, but during the hour of anesthesia the plasma volume gradually approached the control value. A third cat responded to ether first with a decrease in plasma volume amounting to 7.4 per cent of the original value, but at the end of 40 minutes the plasma volume had been restored to its control level, and after 20 minutes more an 8 per cent increase had occurred. The early hemoconcentration in this experiment cannot be attributed to muscular activity during the induction period. The cat was remarkably quiet and was anesthetized with absolutely no struggling.
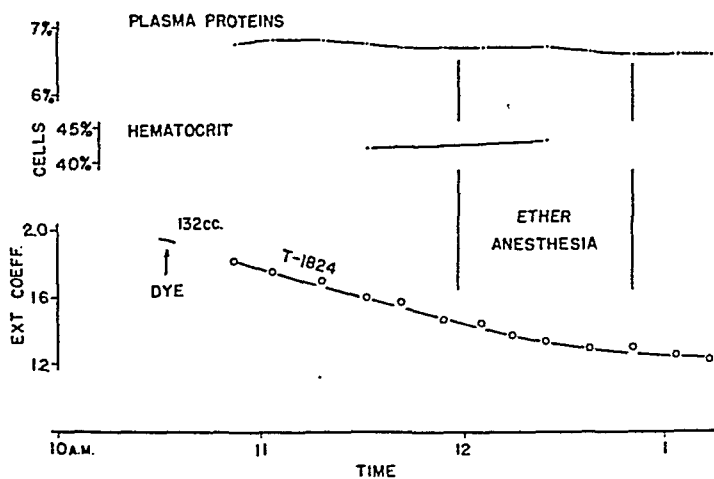
Fig. 1. Experiment on cat 10, weight 3.30 kgm. (6/27/39). Ether was administered for 50 minutes at the time indicated. The gradual flattening of the dye concentration curve is usually seen in unanesthetized cats and is a result of a decrease in the disappearance rate of the dye. No change in plasma volume is indicated by either the dye or protein concentration curves, while the hematocrit shows a slight but hardly significant rise.

In those experiments in which hematocrits were determined (table 1), one reading was taken during the pre-anesthetic period and another from 20 to 60 minutes after etherization had begun. One animal (cat 12) showed an increase in hematocrit of 4.7 per cent after 50 minutes of anesthesia, at a time when the dye and protein concentrations indicated that an increase in plasma volume had occurred. The other readings were within 2.5 per cent of the control value.

DISCUSSION. The experiments clearly demonstrate that in the cat ether anesthesia does not cause a decrease in plasma volume. In this respect, therefore, the cat differs from the dog. According to the experiments of McAllister, etherized dogs showed an average reduction in plasma

volume of 11 per cent. The mechanism by which ether anesthesia leads to a significant reduction of the plasma volume of most normal dogs is not thoroughly understood. Root and McAllister (1939, 1940) have shown that the effect may be abolished by sympathectomy or by section of the spinal cord in the cervical or upper thoracic region. The change in plasma volume appears in some way to be dependent upon the integrity of the sympathetic system and its supra-segmental control. Hence it would seem reasonable to suppose that fluid is lost from the plasma as a result of altered pressure relationships in the capillaries incident to vasomotor changes. And yet it is interesting to note that Gregersen (1940) has demonstrated that the reflex vascular adjustments produced by clamping both carotid arteries in unanesthetized dogs fail to alter the plasma volume.

That ether causes a widespread stimulation of the sympathetic nervous system in the cat has been demonstrated by Bhatia and Burn (1933). The experiments of these investigators showed that the site of action of the anesthetic agent is on the central nervous system rather than peripherally. The studies of Root and McAllister indicate that the action of ether on the sympathetic nervous system of dogs is also central. In this regard, therefore, there is nothing at present to suggest that the basic action of ether differs in the two animals. The contrast in the responses of their plasma volumes to ether would seem more likely to depend upon differences in the result of sympathetic stimulation in the two species. Cannon (1939) has called attention to noteworthy variations between cats and dogs in the functions of the sympathetic system. Perhaps the influence of the sympathetic nervous system on the plasma volume is an example of such a species difference. The effect of generalized sympathetic stimulation on the plasma volume of cats has been subjected to study, but there is no agreement as to the result. Freeman (1933) injected adrenalin at a slow and constant rate into cats anesthetized with Dial and found that the plasma volume was markedly reduced. However, Hamlin and Gregersen (1939) repeated these experiments on unanesthetized cats and found that no decrease occurred.

A surprising finding in these experiments was the failure of the hematocrit values to rise sharply during the period of anesthesia. Bhatia and Burn measured spleen volume with a plethysmograph in decerebrate and spinal cats subjected to ether anesthesia. They found that etherization was associated with a prompt decrease in the volume of the spleen. In dogs, Barcroft and Florey (1929) and Hausner et al. (1938) have observed contraction of the spleen during anesthesia with ether. This splenic contraction is partly responsible for the hemoconcentration which occurs in dogs, for after splenectomy the rise in hematocrit reading during ether anesthesia is reduced to about half (Searles and Essex, 1936; McAllister, 1938; Bollman et al., 1938). In the present series, of the 5 cats·on which

hematocrits were determined, the only animal showing a significant increase was also the only animal which gave evidence of a reduction of plasma volume. In these experiments it seems unlikely that a very intense contraction of the spleen could have occurred.

CONCLUSIONS

In normal cats ether anesthesia does not cause the reduction in plasma volume which has been reported in dogs and in man. The sharp rise in hematocrit reading seen in etherized dogs is also absent in the cat. The suggestion is offered that the differences in these responses of cats and dogs to ether may be attributed to differences in the action of their sympathetic nervous systems. These experiments again emphasize the danger of applying knowledge gained from one species to animals of another.

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RELATION OF SERUM AND MUSCLE ELECTROLYTE, PARTICULARLY POTASSIUM, TO VOLUNTARY EXERCISE[1]

HERBERT C. MILLER AND DANIEL C. DARROW

From the Department of Pediatrics, Yale University School of Medicine

Since abnormal variations of potassium in the extracellular fluid are considered to exert a profound toxic effect on the heart, it is desirable to know if variations of potassium in extracellular and intracellular compartments limit the capacity of skeletal muscle to perform work. In a previous communication we demonstrated that within wide limits the amount of potassium in skeletal muscle did not effect the response of striated muscle to a tetanic stimulus (1). Since the testing of a muscle by a tetanus is not a satisfactory method of estimating the capacity of muscle for work, a less artificial method of testing work capacity was sought. In the present experiments the potassium of the skeletal muscle of rats was varied through wide limits by various means and the rat's ability to work was tested by having the animal swim continuously. It is obvious that in such experiments a failure to swim cannot necessarily be attributed to a decrease in muscle function, since many other factors might equally well explain the failure. If satisfactory work performances could be obtained in spite of abnormal variations of potassium in extracellular and intracellular fluids of the skeletal muscle, then an altered potassium in these fluids could not be accepted as an explanation for the failures.

METHODS. Adult male rats weighing between 250 and 425 grams were used in all experiments. They were fed either Purina Dog Chow (stock diet) or a "low K" diet previously described (1). Food and water were given freely at all times. All rats were in apparent good physical condition at the time they swam.

The rats swam in a galvanized iron tank measuring 26 inches high by 20 inches in diameter filled with water at 37°C. to within four inches of the top. Since with no weight attached rats frequently could "loaf" and float, a five gram weight was attached by an elastic band to the base of the tail. The elapsed time each rat spent in the water was noted. A swimming performance was considered satisfactory if the animal swam con-

tinuously for 60 minutes.   Some rats were permitted to swim to the point of exhaustion.   Exhaustion was determined by the inability of the rat to reach the surface of the water although it was making every attempt to do so.   Occasionally rats submerged at the bottom for several seconds, evidently seeking a means of escape.   By careful observation it was not difficult to determine when submersion was due to exhaustion and when it was due to other factors.   Immediately upon termination of the swim the rat was etherized and blood withdrawn under oil from the abdominal aorta.   Analyses of serum and muscle were made in the manner described in a previous communication (2).   The muscles of the hind legs were the only ones used in the present study.   The hearts of several rats were removed for pathological examination.   Histological preparations were

TABLE 1

Serum and muscle electrolyte in exercised and resting rats with normal muscle potassium

GROUP	DIET	NUM-BER OF RATS	AVER-AGE TIME SWAM	SERUM			MUSCLE PER 100 GM. FAT-FREE SOLIDS					
				Per L. serum ultrafiltrate		Per L. serum	Na	Cl	K*	P	Prot.	H₂O
				Na	Cl	K						
			minutes	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*gm.*	*cc.*
I	Stock	9	47	148.9 ±0.99	113.2 ±0.99	4.7 ±0.22	11.5 ±0.45	8.3 ±0.31	49.0 ±0.45	33.3 ±0.33	96.3 ±0.95	366.6 ±2.02
IA	Stock	13	0	146.9 ±0.74	113.0 ±0.87	4.0 ±0.21	9.99 ±0.18	7.2 ±0.15	48.8 ±0.18	33.4 ±0.35	92.3 ±0.56	340.5 ±1.90

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

studied after hematoxylin and eosin stains.   Several rats received daily injections of desoxycorticosterone acetate.[2]   The doses are indicated in the tables.

RESULTS.   Table 1 shows the effect of prolonged exercise on the composition of serum and muscle in normal rats.   The rats of group I swam for a period of from 15 to 110 minutes while those of group I A were killed as they were removed from the cages.   In the serum the only significant change is the small increase in serum potassium from 4.0 to 4.7 mM per liter.   In the muscle there is a definite increase in water with exercise. Since the increase in sodium and chloride of the muscle can account for sufficient extracellular water to explain the change in total muscle water, the amount of intracellular water per unit of fat-free solids is not appreciably changed by exercise.   However, the increase in protein indicates a change in the solids—presumably a loss of some labile fat-free solid such

as glycogen.   Since the data show no change in potassium and phosphorus per unit of fat-free solids, the change in solids is accompanied by loss of these elements.

The changes demonstrated in table 1 were apparently not related to the duration of the exercise—at least within the limits studied.   Neither were they related to the apparent degree of exhaustion.

Table 2 shows the analyses of rats in which the muscle and serum potassium have been significantly increased by repeated hourly injections of KCl intraperitoneally.   Analyses of rats in groups II A and III A are of resting rats and have been reproduced from a previous study (3) in order

TABLE 2

Serum and muscle electrolyte in exercised and resting rats with high muscle potassium

GROUP	DIET	NUM-BER OF RATS	AVER-AGE SUR-VIVAL TIME AFTER LAST IN-JECTION	AVER-AGE TIME SWAM	SERUM			MUSCLE PER 100 GM. FAT-FREE SOLIDS					
					Per L. serum ultrafiltrate		Per L. serum	Na	Cl	K*	P	Prot.	H₂O
					Na	Cl	K						
			minutes	*minutes*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*gm.*	*cc.*
II	Stock	7	19	8	145.9 ±3.28	121.3 ±1.48	9.4 ±0.48	8.4 ±0.36	7.7 ±0.17	50.9 ±0.41	33.6 ±0.39	94.4 ±0.43	366.6 ±0.75
II A	Stock	7	24	0	143.4 ±0.54	121.4 ±0.75	7.5 ±0.20	8.2 ±0.26	7.3 ±0.14	51.7 ±0.52	34.5 ±0.29	95.9 ±1.03	353.0 ±1.54
III	Stock	5	92	81	145.0 ±3.11	121.2 ±1.96	6.0 ±0.51	8.3 ±0.36	7.4 ±0.18	50.2 ±0.29	34.4 ±0.27	94.4 ±0.66	353.0 ±2.55
III A	Stock	5	78	0	145.2 ±0.86	120.0 ±1.14	5.5 ±0.23	8.7 ±0.30	7.2 ±0.19	49.5 ±0.36	33.2 ±0.18	94.8 ±1.28	346.3 ±2.82

* Corrected for extracellular K.

All rats in groups II and III injected with a total of 1.2 to 1.3 meq. KCl per 100 grams of rat in 6 hours.

All rats in groups II A and III A injected with a total of 1.1 to 1.4 meq. KCl per 100 grams of rat in 6 to 7 hours.

All concentrations represent mean result plus or minus standard error.

to show that the muscle potassium is significantly increased within 15 to 30 minutes after the last serial injection of KCl (see group II A) and returns almost to normal if the animal is not sacrificed until 60 to 90 minutes after the last injection (group III A).   Groups II and III represent animals treated in a manner corresponding to groups II A and III A respectively except that they were exercised in addition to receiving injections of KCl. All rats in group II were rapidly exhausted by their swimming.   The fact that they were exhausted did not apparently alter their electrolyte from that of the resting rats in the corresponding group II A.   The only significant changes in electrolyte of the exercised rats of groups II and III from that of the resting rats in groups II A and III A are an increase in muscle water in both of the former and a higher concentration of serum potassium in group II than in II A.   Unlike normal exercised rats there is no increase

in muscle sodium and chloride in the rats in groups II and III over those of the resting rats in groups II A and III A.

The rapidity with which injected potassium leaves the muscle, as demonstrated in table 2, makes it impossible to swim rats for 60 or more minutes and obtain values for muscle potassium at the highest levels. While the muscle potassium of rats in group III is significantly higher than in normal uninjected rats (see group I A), it is fairly certain that during the early part of their swim it was even higher, probably on the order of that of rats in group II. It is unlikely, therefore, that the high muscle potassium of rats in group II accounts for their failure to swim more than eight minutes. It is to be noted that the serum concentration of potassium of rats in group II is at the level at which electrocardiographic changes and toxic effects of potassium on the heart have been noted in cats (4). Presumably the concentration of potassium in the serum of rats in group III would have been found at or near this level had they been sacrificed earlier. Whether the rapid exhaustion of rats in group II was associated with a greater susceptibility to the toxic effects of potassium at this high serum concentration is a matter for conjecture and can not be ascertained from the data at hand. For the purposes of this study it is significant that satisfactory muscular work can be done in the presence of a high muscle potassium.

Owing to the relatively rapid fluctuations of potassium in the muscle when KCl is injected intraperitoneally it was not possible to ascertain what effect, if any, exercise may have on an already abnormally increased potassium in the muscle.

In order to determine if rats with abnormally low muscle potassium i.e., below 44 mM per 100 grams of fat-free solids, were able to swim satisfactorily, two methods for decreasing the muscle potassiums were used: the feeding of a diet low in potassium (3) and the injection of desoxycorticosterone acetate. We also sought to determine if exercise altered the muscle potassium at this low range. Consequently exercised rats that were fed the low K diet, group IV, have been compared with a similarly treated group of rats that were not exercised, group IV A. Similarly the rats injected with desoxycorticosterone acetate were divided into an exercised group, group V, and a resting group, group V A. The results are shown in table 3.

The rats in groups IV and V demonstrate that a low muscle potassium is not in itself a limiting factor in the satisfactory performance of work. Three of the seven rats in group IV swam a full 60 minutes without showing marked evidence of fatigue. The lowest muscle potassium observed (36.9 mM) was in a rat that swam 60 minutes. Comparison of the analyses of exercised rats in group IV with resting rats in group IV A shows that the muscle potassium per unit of fat-free solids apparently remains unchanged with exercise while a slight but significant increase in the concen-

tration of potassium in the serum of the exercised rats occurs. The increases in chloride and water of the exercised rats in group IV over those in group IV A are similar to those observed in exercised rats with normal muscle potassium (see group I) and apparently represent an increase in extracellular fluid in the muscle. The increase in muscle sodium, which was found to be significant in exercised rats in group I, is not so striking in the rats of group IV.

The effect of injecting fairly large amounts of desoxycorticosterone acetate subcutaneously into normal rats, group V A, is to decrease muscle

TABLE 3

Serum and muscle electrolyte in exercised and resting rats with low muscle potassium

GROUP	DIET	NUMBER OF RATS	AVERAGE TIME SWAM	SERUM			MUSCLE PER 100 GM. FAT-FREE SOLIDS					
				Per L. serum ultrafiltrate		Per L. serum	Na	Cl	K*	P	Prot.	H₂O
				Na	Cl	K						
			minutes	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*gm.*	*cc.*
IV	Low K 14 days	7	43	147.3 ±1.63	114.6 ±1.74	4.91 ±0.22	12.5 ±0.59	7.1 ±0.28	39.9 ±0.79	32.3 ±0.28	95.5 ±0.74	349.4 ±4.22
IVA	Low K 14 days	5	0	142.0 ±2.12	113.8 ±0.66	3.96 ±0.16	12.1 ±0.15	6.0 ±0.11	39.8 ±0.36	30.9 ±0.23	94.4 ±0.71	325.0 ±1.76
V	Stock	6	60	149.5 ±3.94	111.1 ±2.23	2.6 ±0.19	14.5 ±0.61	6.6 ±0.45	42.9 ±0.69	32.7 ±0.57	96.8 ±1.39	353.0 ±3.45
VA	Stock	11	0	152.0 ±1.30	107.9 ±1.48	3.7 ±0.08	14.6 ±0.39	6.3 ±0.08	40.5 ±0.82	31.7 ±0.24	95.5 ±0.59	328.0 ±3.59

* Corrected for extracellular K.

Group V—injected daily for 9 days subcutaneously with 2 mgm. desoxycorticosterone acetate in 25 per cent glucose.

Group VA—eight rats injected same as group V; remaining three rats injected with 3 to 4 mgm. desoxycorticosterone acetate in oil daily for 10 days.

All concentrations represent mean result plus or minus standard error.

potassium without altering the concentration of serum potassium. With the decrease in muscle potassium there is an increase in muscle sodium similar to that noted in rats fed a diet low in potassium (3). Both the serum and muscle chloride are significantly decreased. There is also a loss of muscle water. Contrary to the results obtained in rats fed the "low K" diet or the stock diet the muscle potassium increases and the concentration of potassium in the serum decreases with exercise (see group V). The changes in muscle sodium and chloride in the exercised rats of group V are negligible. The increase in muscle water, however, is significant. The failure to demonstrate an increase in muscle sodium in the rats in group V over that of the resting rats in group V A may have been

dependent on the fact that the expected increase associated with exercise was offset by the decrease in this cation that accompanies an increase in muscle potassium. It is to be noted that the increase in the muscle potassium of rats in group V is an absolute increase, since no change in muscle protein was observed over the control group V A. The work performance of the rats in group V was entirely satisfactory even though the muscle potassiums were below the recognized lower limit of the normal range (44 to 50 mM per 100 grams of fat-free solids).

Efforts were made to obtain satisfactory swimming performances with rats that had even lower muscle potassiums than those observed in table 3. Previous work had demonstrated that muscle potassiums as low as 30 mM per 100 grams of fat-free solids could be obtained by feeding rats on a low K diet for many weeks (3). Consequently a group of 7 rats that had subsisted on the diet low in potassium from 32 to 133 days were made to swim. One rat that had been on the diet 32 days swam for 52 minutes without fatigue, but all five of the rats that had been fed on the diet longer than 42 days became exhausted from swimming in 7 minutes or less. While this group of rats had muscle potassiums down to 30 mM in some instances, it is much more likely that pathological changes in the cardiac muscle accounted for their failure to swim well rather than the low muscle potassium. Microscopic studies[3] of the hearts of several of the rats that were so readily exhausted were made and pathological changes were found to be present. These changes correspond to those previously described by Thomas et al. in animals fed a low potassium diet (5).

Very low muscle potassium was also produced in seven rats by daily subcutaneous injection of 4 mgm. of desoxycorticosterone acetate for 30 days. The compound was suspended in a 10 per cent solution of glucose. The diet (Purina Dog Chow) contained adequate potassium. Three of the four rats which were placed in the tank were unable to swim longer than 20 minutes. The other one swam 60 minutes without apparent exhaustion. This rat showed a muscle potassium of 33.2 mM per 100 grams of fat-free solids, the lowest value found in any rat able to swim adequately. Serum potassium was 3.3 mM per liter.

The hearts of the rats given the excessive doses of desoxycorticosterone acetate were examined histologically except, unfortunately, the one which swam adequately. In each case, the myocardial fibres showed fairly extensive necrosis with displacement of necrotic fibres by connective tissue. The latter feature was not prominent since the necrosis apparently was not very old. The areas involved tended to be near larger blood

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[3] We are indebted to Drs. M. C. Winternitz and H. M. Zimmerman of the Department of Pathology for their interpretation of the microscopic studies of the hearts of the animals fed the low K diet and also those injected with desoxycorticosterone acetate.

vessels but could be found in all parts of the heart.   The lesions seemed to be comparable to those seen in rats fed a diet low in potassium for several weeks.   Two of the rats which failed to swim showed ascites.   It was felt that myocardial failure explained the inability of rats injected with excessive doses of desoxycorticosterone acetate to swim adequately.

Discussion.   The variations in the period of the swim of normal rats were found to be quite large.   In two instances apparently normal rats with normal muscle potassium were exhausted within 10 minutes, two others were fatigued after 43 and 75 minutes and one rat swam 110 minutes without apparent fatigue.   It seems justified to assume that 60 minutes of continuous swimming indicates a satisfactory performance.

The experiments clearly show that certain rats can swim for one hour with either a low or a high muscle potassium.   Individual values as low as 33.2 and as high as 50.8 mM per 100 grams of fat-free solids were obtained at the conclusion of a swim of 60 minutes.   Previous work showed that the muscle potassium of normal resting rats may vary from 44 to 50 mM per 100 grams of fat-free solids without being accompanied by a gross disturbance in either the serum or muscle electrolytes (3).   While 50.8 mM of potassium per 100 grams of fat-free solids was the highest recorded value in a rat with a satisfactory swimming performance, the results in table 2 indicate that successful swimming was done by rats with considerably higher muscle potassiums.   Although the muscle potassium of rats in group III was maintained at abnormally high levels for probably only 30 to 60 minutes, the results suggest that the similarly high muscle potassiums of rats with advanced adrenal insufficiency (2) are not per se the cause of the rapid muscle fatigue and weakness seen after adrenalectomy (6).   While moderately low muscle potassiums, about 40 mM per 100 grams of fat-free solids, are compatible with satisfactory swimming performances in most instances, it is only rarely that rats can swim 60 minutes with much lower muscle potassiums.   The myocardial injury associated with the very low potassium in the skeletal muscle, whether produced by a low K diet or the injection of desoxycorticosterone acetate, probably is the limiting factor.

Attention is directed to the fact that adequate muscular performances were obtained in group V although the concentration of potassium in the serum was very low (1.9 to 3.1 mM per liter).   These rats received desoxycorticosterone acetate in amounts and over periods similar to the dogs reported by Kuhlman et al. (7).   Our results suggest that myocardial injury rather than an abnormally low concentration of potassium in the serum is the cause of the "paralysis" and weakness noted in otherwise normal animals treated with desoxycorticosterone acetate.

The fluctuations in the level of serum potassium are of interest since these changes are sometimes used to indicate loss of muscle potassium

during exercise. Except in rats receiving desoxycorticosterone acetate, the exercising rats show a rise in serum potassium similar to that reported previously (8). Our data show that if potassium is lost from the muscle during exercise in the intact rat, it is lost together with sufficient fat-free solids to leave the potassium unchanged per unit of fat-free solids. Although we have no direct proof that non-protein fat-free solids were lost during exercise in the present experiments, such an occurrence would best explain the relative increase in protein per 100 grams of fat-free solids equivalent to four grams in group I. Expressing the relative increase in protein in terms of fat-free tissue (i.e., wet weight), the change in protein suggests a loss of fat-free solids equivalent to 0.5 per cent of the fat-free muscle. Within a few minutes after exercise, Flock and Bollman have demonstrated a loss of glycogen of about 0.3 to 0.4 per cent (9). If this glycogen is released together with sufficient potassium to keep the potassium constant per unit of fat-free solids, an exercising rat weighing 300 grams might free as much as 0.066 mM of potassium. This amount of potassium would raise the concentration of serum potassium 0.8 mM if confined to extracellular water and 0.3 mM if equally distributed throughout body water. Potassium might also appear in the serum from the liver since Fenn (10) has shown that glycogen is deposited in the liver together with potassium. Since the liver weighs about 8 grams the loss of 2 per cent glycogen would free about 0.02 mM of potassium. It is obvious that these two sources of potassium are more than sufficient to explain the rise in serum potassium accompanying exercise.

Fenn has postulated that muscular contraction is accompanied by a loss of potassium from the muscle per unit of solids (8). There can be no doubt, as Fenn points out, that electrical stimulation of an isolated muscle or group of muscles in an otherwise intact animal leads to a loss of potassium and a gain of sodium. Evidence that similar changes accompany *voluntary* contraction is not satisfactory. The only direct evidence for this view is submitted by Fenn (11). His conclusions are based essentially on differences between innervated and denervated muscle in resting as well as swimming rats. His data indicate that the "loss of potassium" with voluntary exercise may be due largely to a gain of potassium by the denervated muscle rather than to an actual loss from innervated muscle. To avoid this difficulty it would seem better to compare the muscles of rats that swam to those of rats confined to cages. Although the potassium of the innervated muscle from the exercised rats is lower than that of the resting rats, the difference is not statistically significant. The present experiments indicate that voluntary contraction is not accompanied by a loss of potassium beyond that associated with loss of glycogen or other fat-free solids. In fact, our work with rats injected with desoxycorticosterone acetate shows that potassium actually can enter the skeletal muscle cells during voluntary exercise.

SUMMARY

Within wide limits the amount of potassium in the muscle cells does not limit the capacity of rats to swim continuously for sixty minutes.

Concentrations of potassium in the serum that are abnormally low are not in themselves sufficient to produce "paralysis" or muscular weakness in normal animals.

No loss of potassium per unit of fat-free solids from striated muscle could be demonstrated after relatively long periods of voluntary exercise. An absolute increase in muscle potassium was demonstrated in exercised rats injected with desoxycorticosterone acetate.

Desoxycorticosterone acetate decreases the muscle potassium and increases muscle sodium in the normal resting rat. These changes involve an apparent replacement of intracellular potassium by sodium.

The increase in the concentration of potassium in the serum immediately following exercise was confirmed. The possible sources of this increase were discussed. The concentration of potassium in the serum of exercised rats injected with desoxycorticosterone is decreased.

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